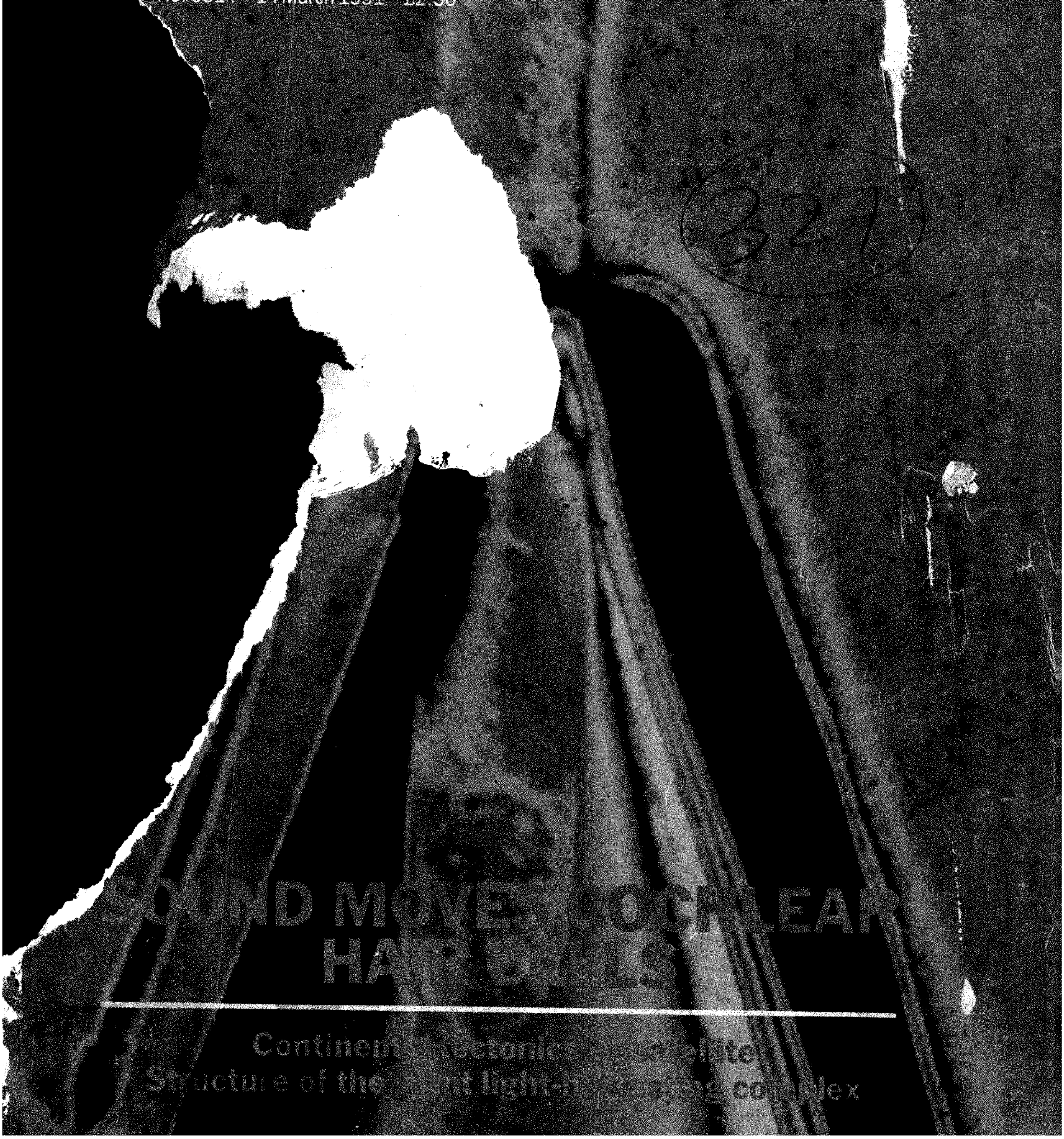
[illegible] 327 [illegible]

nature

ERNATIONAL WEEKLY JOURNAL OF SCIENCE

No. 6314 14 March 1991 £2.50

327



SOUND MOVES COCHLEAR HAIR CELLS

Continental tectonics by satellite
Structure of the plant light-harvesting complex

Are there GMP peptides in your future?

A dominant supplier of research-quality peptides, Multiple Peptide Systems is also an expert in large-scale and bulk pharmaceutical peptide manufacturing. Consider MPS as your source for GMP peptides.

Multiple Peptide Systems is one of a select few companies licensed to manufacture bulk pharmaceutical peptide products for *both* drug and device applications. MPS will work with you, in strict confidence, from initial clinical trials through final production.

Licensed by the California Department of Health Services, MPS is registered with the FDA to produce peptides under current Good Manufacturing Practices (cGMP).

Our manufacturing and quality control labs, combined with our rigorous documentation system, ensure the high quality of your products in bulk pharmaceutical quantities. When filing your DMF, ELA, PLA, and other regulatory documents, Multiple Peptide Systems will assist you through the long and sometimes difficult process of product development and licensing. We invite you to visit and inspect our facilities.

PARTIAL VIEW OF A GMP LABORATORY AT MULTIPLE PEPTIDE SY

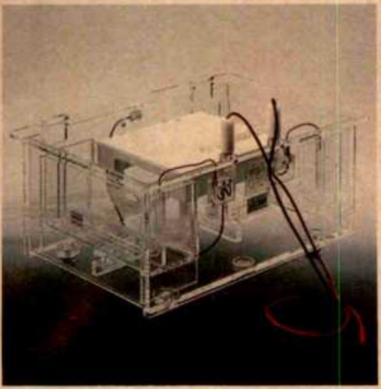
Multiple Peptide System

3550 General Atomics Court, San Diego, CA 92121 USA
Tel. (619) 455-3710
Toll Free (800) 338-4965
FAX (619) 455-3713
Toll Free FAX (800) 654-5592

EUROPEAN DISTRIBUTORS
Switzerland
NOVABIOCHEM AG
Tel. (062) 69.19.22
Italy
INALCO SpA
Tel. (02) 55213005
France
EMA
Tel. (1) 34.67.44.44
Germany
NOVABIOCHEM GmbH
Tel. (06224) 54031
United Kingdom
NOVABIOCHEM Ltd.
Tel. (0602) 430840

Reader Service No. 621

- Quantitative PCR
- Detection of PCR artifacts
- Preparation of mutants



The TGGE system

- superimposes a temperature gradient on the electrical field
- separates nucleic acid fragments of identical length by their sequence properties

TGGE - PCR applications

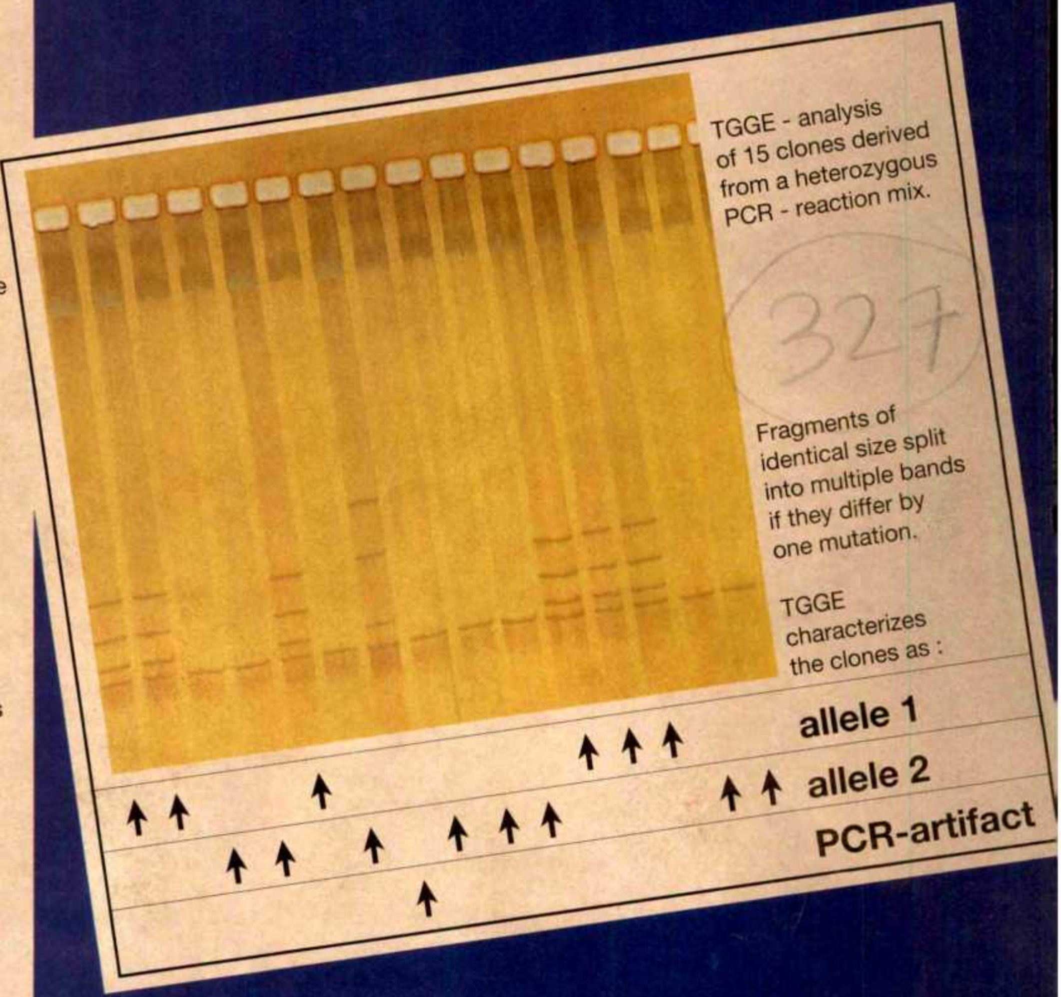
- confirmation of the accuracy of your PCR amplified reaction mixture
- direct analytical detection and preparative separation of polymorphic alleles from a PCR amplified reaction mixture
- check for PCR induced mutations in cloned sequences without sequencing procedures
- reliable determination of PCR-template concentration, independent of the number of cycles, of plateau effects, of unbalanced primer concentration or, of variable PCR reaction kinetics

TGGE features

- results in less than 5 hours
- screening of multiple samples on one gel
- many lab-tested protocols
- denaturing temperature gradients with high reproducibility

DIAGEN supplies

- the complete TGGE system
- a large variety of application protocols



TGGE - analysis of 15 clones derived from a heterozygous PCR - reaction mix.

327

Fragments of identical size split into multiple bands if they differ by one mutation.

TGGE characterizes the clones as :

**Temperature
Gradient
Gel
Electrophoresis System**

The Polymerase Chain Reaction (PCR) is covered by U.S. Patent No. 4.683.202 issued to Cetus Corp.. Patents are pending in other countries.

USA/CANADA: QIAGEN Inc., Chatsworth, CA 91311, Phone (800) 426-8157, (818) 718-9870, Fax (818) 718-2056
GERMANY: DIAGEN GmbH, Niederheider Str. 3, D-4000 Düsseldorf 13, Phone (0211) 79 30 37, Fax (0211) 79 04 44
DISTRIBUTORS: AUSTRALIA: Phoenix Stansens Scient.Div. (3) 544 8022 **AUSTRIA:** Bio-Trade (222) 828 46 94
BENELUX: Westburg B.V. (NL-33) 95 00 94 **FRANCE:** Coger (1) 45 33 67 17 **ISRAEL:** Bio-Lab Laboratories Ltd.
(2) 52 44 47 **ITALY:** Genenco (M-Medical) (55) 67 64 41 **JAPAN:** Funakoshi Co., Ltd. (3) 5684 1622
KOREA: LRS 924-8697 **PORTUGAL:** Izasa Portugal, S.A. 758 07 40 **SCANDINAVIA:** Kebo Lab: Denmark: (44) 68 18 00,
Finland: (90) 437 56 40, Norway: (02) 30 11 20, Sweden: (08) 621 34 00 **SPAIN:** Izasa S.A. (3) 401 01 01
SWITZERLAND: Kontron Instruments AG (1) 733 5 733 **TAIWAN:** Formo (2) 736 7125 **UK:** Hybaid Ltd. (81) 977 3266

DIAGEN

Reader Service No 564

Biometra® presents:

TRIO – Thermoblock

3 INDEPENDENTLY DRIVEN THERMOBLOCKS IN ONE INSTRUMENT

- immediate processing of finished samples while others are still under preparation
- up to 60 samples of 0.5 mls in 3 blocks with superior block temperature uniformity
- microprocessor controlled Peltier technique for ultimate temperature accuracy
- cooling of samples possible at any stage of experiment
- rapid temperature changes for maximum saving of time
- 12 variable programmes with macro functions for complex cyclic and non-cyclic temperature profiles

Get your 3 blocks for the price of one

Austria: Chem. Laboratorium Gatt, phone 512-583017
Belgium: Sopar-Biochem, phone 02-425-1455
Canada: Emerston Instruments Inc., phone 416-886-9440
France: Bioblock Scientific, phone 88671414
Eurogentic S.A., phone 41733373
India: Enzomed Biotech, phone 11-6442463
Italy: Vios Instruments S.r.l., phone 02-4151721
Japan: Funakoshi Pharmaceut. Co. LTD, phone 03-5684161
Netherlands: Salm & Kipp, phone 3462-62814
South-Africa: Omni-Science CC, phone 11-708-1912
Spain: TDI, S.A., phone 01-409 1215
Switzerland: Biogenzia Lemania SA, phone 21-254011
Taiwan: Schmidt Scientific, phone 2-5005797
Reader Service No.585

Biometra

Germany (FRG): Rudolf-Wissell-Straße 30 · D-3400 Göttingen · Tel. 05 51/5 06 86-0 · Fax 05 51/5 06 86 66
United Kingdom: P.O. Box 167, Maidstone, Kent Me14 2AT · Tel. 0622-678872 · Fax 0622-7527744
P.O. Box 42, Sale, Manchester M33 5DP · Tel. 061-962-9634 · Fax 061-962-9715

Get the full picture

Markers
First Strand
Second Strand
FULL LENGTH cDNA PRODUCTION USING SuperScript™ • SIZE FRACTIONATION • DIRECTIONAL CLONING OF INSERTS • MULTIFUNCTIONAL VECTORS • PRECUT VECTORS λgt22A OR pSPORT 1 • IPTG INDUCED cDNA EXPRESSION • cDNA CLONES GENERATED • SCREENING & EXPRESSION OF cDNA CLONES
SUPERSCRIPT™ SYSTEMS
GIBCO BRL
a cDNA Production

Alkaline Agarose Gel Analysis of First and Second Strand cDNA Synthesised with the SuperScript™ Plasmid System. Samples of ^{32}P labeled first or second strand cDNA made from Hela mRNA were ethanol-precipitated, dissolved in alkaline agarose sample buffer, and electrophoresed on a 1.4% agarose gel at 15V for 16h.

Full length cDNA production has never been simpler . . .
Life Technologies new SuperScript™ kits are the systems of choice for full length cDNA clone generation and expression.

Flexible Systems:
- 50% Higher yield of full length cDNA
- Size fractionation using pre-packed columns
- Directional insertion of full length cDNA
- Multiple cloning sites
- Choice of vectors: λ gt 22A or pSPORT 1™
- Expression within 4 days

The strong supporting cast includes restriction and modifying enzymes, gel electrophoresis equipment and a wide range of Ultrapure™ reagents. So when you need full length multifunctional cDNA clones FAST, contact Life Technologies and order either the SuperScript™ Plasmid System Cat. No. 530 8248SA or the SuperScript™ Lambda System Cat. No. 530 8256SA.

GIBCO BRL

LIFE TECHNOLOGIES
EUROPEAN DIVISION

United Kingdom Life Technologies Ltd, PO Box 35, Trident House, Renfrew Road, Paisley PA3 4EF, Scotland. Tel: 041 889 6100 Telex: 777968/777029 Fax: 041 887 1167
United Kingdom Life Technologies Ltd, Unit 4, Cowley Mill Trading Estate, Longbridge Way, Uxbridge UB8 2YG, Middlesex, England. Tel: 0895 36355 Fax: 0895 53159
Belgium N.V. Life Technologies S.A., Drongensesteenweg 68, 9000 Gent, Belgium. Tel: 091 27 70 31 Telex: 12101 Fax: 091 27 75 25
Denmark Life Technologies A/S., P.O. Box 198, Industrivej 1, DK-4000 Roskilde, Denmark. Tel: (++45) 46 75 63 11 Telex: 43261 Fax: (++45) 46 75 70 10
France Life Technologies Sarl, 1 Rue du Limousin, BP 7050, 95051 Cergy Pontoise, Cedex, France. Tel: 1 34 64 54 40 Telex: 609098 Fax: 1 30 37 50 07
Netherlands Life Technologies B.V., Hekven 3, 4824, AD Breda, The Netherlands. Tel: 076 41 99 44 Telex: 64016 Fax: 076 41 95 19
Switzerland Life Technologies A.G., Zwingerstrasse 12, CH 4018 Basel, Switzerland. Tel: 061 35 40 66 Telex: 964520 Fax: 061 35 85 92
Germany Life Technologies GmbH, Postfach 1212, Dieselstrasse 5, 7514 Eggenstein, Germany. Tel 0721 780 40 Telex: 7825957 Fax: 0721 780 499
Germany Life Technologies GmbH, Innsbruckerstrasse 25, 1000 Berlin 62, Germany. Tel: 030 8 53 2088, 030 8 53 6095 Fax: 030 8 53 6096
Reader Service No.579

ECL

Light-based detection for Western blots

Trying to choose between ^{125}I, colorimetric and other non-radioactive systems?

With the new ECL Western blotting system, which complements our other systems, we can now help you choose the technology, radioactive or non-radioactive, to best suit your experimental needs.

The ECL (enhanced chemiluminescence) system is based on using horseradish peroxidase labelled second antibodies, or biotin-streptavidin systems.

In the presence of the ECL Western blotting detection system an enhanced light signal is created.

- 10 times as sensitive as colorimetric systems
- Results in minutes, often seconds
- Economical use of primary and secondary antibodies
- Simple protocols
- Carry out normal blotting and immunodetection techniques and then add ECL detection system for 1 minute
- No fading of results; hard copy results on film

ECL Western blotting detection system RPN 2106
(sufficient for 4000cm^2 of blots)

Contact your local Amersham office for further details

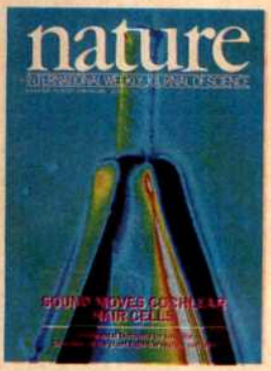
Amersham International plc
Amersham UK

Amersham
Bringing science to life

UK Sales Aylesbury (0296) 395222 **Amersham Australia Pty Ltd** Sydney (02) 888-2288 **Amersham Belgium SA/NV** Brussels (02) 770 0075
Amersham Buchler GmbH & Co KG Braunschweig West Germany (0 53 07) 2 06-0 **Amersham Canada Ltd** Oakville, ONT (416) 847-1166
Amersham Corporation Arlington Heights, IL USA (800) 323-9750 **Amersham Denmark ApS** Birkerød 45-82 02 22 **Amersham France SA** Les Ulis (1) 69 86 53 00
Amity Milan, Italy 2-5097720 **Amersham Japan** Tokyo (03) 816 6161 **Amersham Nederland BV** 's-Hertogenbosch 073-41 82 25
Amersham — Norway branch Gjettum 02-54 63 18 **Amersham Sweden AB** Solna 08-734 08 00

Reader Service No.5

P 36666



nature
14 March 1991
Vol. 350 Issue no.6314

◀ Outer hair cells are known to act as sensory receptors, but they may also have a role as a mechanical feedback device. Cover shows an isolated cochlear outer hair cell half-way inserted into a tight-fitting pipette so that the cell is partitioned both mechanically and electrically. Dallos *et al.* on page 155 show that the cell's motility is driven by local changes in transmembrane potential, rather than by mechanisms of electrophoresis or electroosmosis.

THIS WEEK . . . THIS WEEK . . . THIS WEEK . . .

On the move

Triangulation data obtained in 1890 from fifteen sites in central Greece have been compared with up-to-date information from the Global Positioning System of navigational satellites to yield a measure of the extent of deformation in this region of the Earth's crust over a 100-year period. High-precision GPS surveys such as this show promise as a component in earthquake hazard assessment. Page 124.

Seeing the light

The stucture of a 'light-harvesting' complex of a higher plant has been determined by electron crystallography, revealing the arrangement of protein and chlorophyll in the transmembrane complex. Pages 130 and 109.

Heavy metal

X-ray imaging of the Virgo cluster of galaxies reveals a spatial gradient in iron concentrations. Heavy elements such as iron are synthesized in stars, and are not present in primordial matter, so the presence of this iron gradient gives a picture of the distribution of 'old' and 'new' matter in an evolving galactic cluster. Page 135.

Ball hunt

Mary Evans

Salagnac, France, 1884. Ball lightning kills a pig.

What causes ball lightning? There is no generally accepted theory, but laboratory experiments by Ohtsuki and Ofuruton produce fireballs exhibiting properties similar to those reported for natural ball lightning, and lend some support to a theory put forward by Kapitza in 1955. Pages 139 and 108.

Water works

Water will not normally 'wet' the surface of rubbery materials, but a new form of latex has the unusual property of being water-wettable. Applications of such technology might begin with bandages and disposable nappies (diapers). Pages 143 and 112.

NGF transduction

Stimulation of neural cell lines with nerve growth factor is known to result in phosphorylation of tyrosine on various unidentified proteins. Kaplan *et al.* (page 158) show that one of the proteins is the product of the *trk* oncogene, suggesting that this protein may be a component of the elusive high-affinity NGF receptor.

Rapid reaction

Triosephosphate isomerase is 10^{10} times more effective at catalysing its target reaction than a 'conventional' catalyst. In this week's Review Article, Jeremy Knowles takes this enzyme as an example in assessing the progress in our understanding of the nature of enzyme catalysis. Page 121.

Resilient mosquitoes

A detailed molecular analysis of the amplified esterase B genes responsible for organophosphate insecticide resistance in mosquitoes suggests that these genes have been spread worldwide following an initial amplification event. Pages 151 and 107.

Chimaeric algae?

The discovery of two different transcriptionally active eukaryotic 18S ribosomal RNA genes in the total genome of a cryptomonad alga suggests that cryptomonad chloroplasts evolved from a photosynthetic eukaryote endosymbiont. Furthermore, the chloroplasts of the different photosynthetic groups appear not to be the result of a single endosymbiont event. Page 148.

Guide to Authors

Facing page 121.

CONTENTS

NATURE SAYS

Forensic science questioned ■ Telecommunications liberalizations should be more radical 95–96

NATURE REPORTS

New contributions to Human Frontiers ■ Europe and the LHC ■ Amgen wins patent ■ Scientific misconduct and NIH ■ Predicting earthquake risks 97–103

CORRESPONDENCE

Runaway complexity ■ Origins of AIDS ■ Etc 104

NEWS AND VIEWS

Classical liquid in a box
John Maddox 105
Eukaryote origins: Seeds of a universal tree
David Penny & Charles J O'Kelly 106
Molecular evolution: The resistance movement
J F Y Brookfield 107
Ball lightning: Great balls of fire
Stanley Singer 108
Photosynthesis: Absorbing developments
M C W Evans & J H A Nugent 109
Milky way: Not with a whimper
Leo Blitz 110
Bone marrow transplantation: Making just the right match
Peter Parham 111
Obituary: Salvador E Luria
James D Watson 113
Optical communications: As quick as a flash
Paul Calvert 114
Daedalus: Food on the hop 114

SCIENTIFIC CORRESPONDENCE

Deficiency in red blood cells D MacDonald, M Town, P Mason, T Vulliamy, L Luzzatto & D K Goff
■**Sea-level constraints** K Lambeck & M Nakada 115
Another recipe for bomb ^{14}C dilution G H Rau 116

BOOK REVIEWS

Preventing a Biological Arms Race *by S Wright*
Alastair Hay 117
R & D for Industry — A Century of Technical Innovation at Alcoa *by M B W Graham & B H Pruitt*
Robert W Cahn ■ **Confocal Microscopy** *ed T Wilson*
Graham Dunn 118
Black Holes and the Universe *by I Novikov*
John L Friedman 119
Human Change Processes: The Scientific Foundations of Psychotherapy *by M J Mahoney* Stuart Sutherland 120

REVIEW ARTICLE

Enzyme catalysis: not different, just better
J R Knowles 121

ARTICLES

Geodetic determination of tectonic deformation in central Greece from 1900 to 1988
H Billiris, D Paradissis, G Veis, P England, W Featherstone, B Parsons, P Cross, P Rands, M Rayson, P Sellers, V Ashkenazi, M Davison, J Jackson & N Ambraseys 124►

Nature® (ISSN 0028-0836) is published weekly on Thursday, except the last week in December, by Macmillan Magazines Ltd (4 Little Essex Street, London WC2R 3LF). Annual subscription for USA and Canada US$350 (institutional/corporate), US$135 (individual making personal payment). USA and Canadian orders to: *Nature*, Subscription Dept, PO Box 1733, Riverton, NJ 08077-7333, USA. Other orders to *Nature*, Brunel Road, Basingstoke, Hants RG21 2XS, UK. Second class postage paid at New York, NY 10012 and additional mailing offices. Authorization to photocopy material for internal or personal use, or internal or personal use of specific clients, is granted by *Nature* to libraries and others registered with the Copyright Clearance Center (CCC) Transactional Reporting Service, provided the base fee of $1.00 a copy plus $0.10 a page is paid direct to CCC, 27 Congress Street, Salem, MA 01970, USA. Identification code for *Nature*: 0028-0836/91 $1.00 + $0.10. US Postmaster send address changes to: *Nature*, 65 Bleecker Street, New York, NY 10012. Published in Japan by Nature Japan K.K., Shin-Mitsuke Bldg, 36 Ichigaya Tamachi, Shinjuku-ku, Tokyo 162, Japan. © 1991 Macmillan Magazines Ltd.

Primer Specificity Breakthrough.

Perfect Match™ Polymerase Enhancer

Dramatically Reduces False Priming • Enhances Specificity of PCR Amplification

Perfect Match™ polymerase enhancer* is an additive formulated to greatly increase the specificity of primer extension reactions (1,2). Primer-template complexes that contain mismatched nucleotides near the 3′ terminus of the primer are destabilized by Perfect Match polymerase enhancer (3). However, perfect and near perfect template-primer matches or primers with regions of non-homology at the 5′ end are not affected. Thus, the frequency of false priming events is dramatically decreased. Perfect Match polymerase enhancer may be used to minimize false priming events in such applications a cDNA synthesis and primer extension reactions including those involving PCR amplification (4).

1. Nielson, K. and Mathur, E.J. (1990) Strategies 3:17-19.
2. Nielson, K. and Mathur, E.J. Manuscript in preparation.
3. Nielson, K. and Mathur E.J. (1989) U.S. patents filed.
4. Mullis, K.B., and Faloona, F.A. (1987) Meth. Enzymol. 155:335-350.

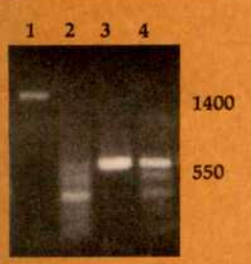
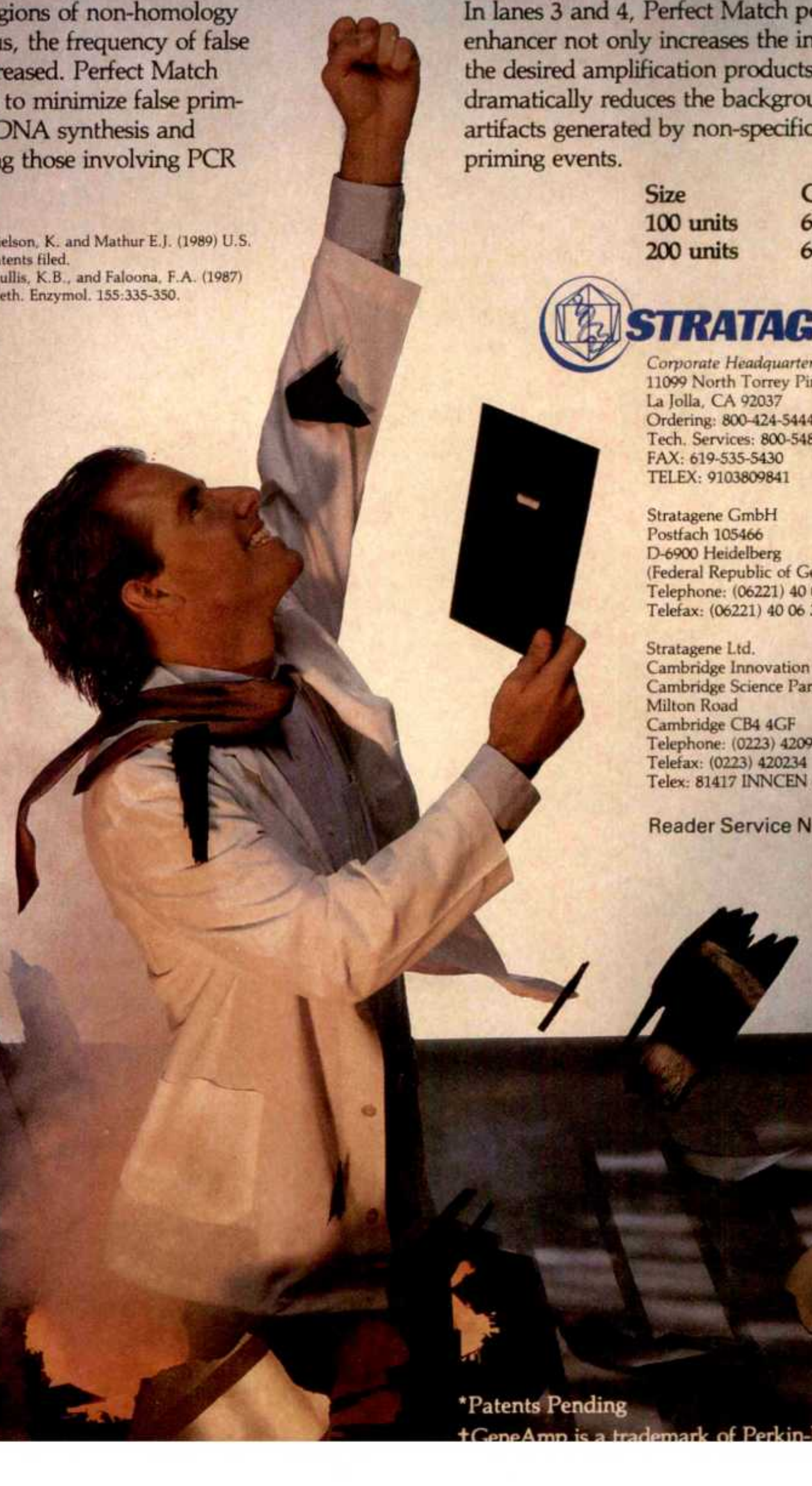


Figure Legend: A photograph of a 1% agarose gel stained with ethidium bromide representing reaction products from PCR amplifications using the GeneAmp™ Kit† from Perkin-Elmer Cetus according to manufacturer's instructions. The reactions were conducted with (lanes 1 and 3) and without (lanes 2 and 4) the inclusion of 1 unit Perfect Match polymerase enhancer. Lanes 1 and 2 represent 100 ng of human genomic DNA amplified with two 26-mer primers separated by 1400 nucleotides. Lanes 3 and 4 represent 100 ng of mouse genomic DNA amplified with two 23-mer primers separated by 550 nucleotides.

Figure 1 shows two examples of *in vitro* amplification reactions that are significantly enhanced by the addition of Perfect Match polymerase enhancer to the polymerase preparation. Note that in lanes 1 and 2, the desired PCR product cannot be detected unless Perfect Match polymerase enhancer is added to the amplification reaction. In lanes 3 and 4, Perfect Match polymerase enhancer not only increases the intensity of the desired amplification products, but dramatically reduces the background artifacts generated by non-specific priming events.



Size	Catalog #
100 units	600129
200 units	600130

STRATAGENE®

Corporate Headquarters:
11099 North Torrey Pines Road
La Jolla, CA 92037
Ordering: 800-424-5444
Tech. Services: 800-548-1113
FAX: 619-535-5430
TELEX: 9103809841

Stratagene GmbH
Postfach 105466
D-6900 Heidelberg
(Federal Republic of Germany)
Telephone: (06221) 40 06 34
Telefax: (06221) 40 06 39

Stratagene Ltd.
Cambridge Innovation Centre
Cambridge Science Park
Milton Road
Cambridge CB4 4GF
Telephone: (0223) 420955
Telefax: (0223) 420234
Telex: 81417 INNCEN G

Reader Service No.670

*Patents Pending
†GeneAmp is a trademark of Perkin-Elmer Cetus

Three-dimensional structure of plant light-harvesting complex determined by electron crystallography
W Kühlbrandt & D N Wang N&V 130

LETTERS TO NATURE

Iron distribution in the intracluster gas of the Virgo cluster of galaxies
K Koyama, S Takano & Y Tawara 135

Irradiation-driven mass transfer in low-mass X-ray binaries
Ph Podsiadlowski 136

Plasma fireballs formed by microwave interference in air
Y H Ohtsuki & H Ofuruton N&V 139

The 154-day and related periodicities of solar activity as subharmonics of a fundamental period
T Bai & P A Sturrock 141

Latex elastomer with a permanently hydrophilic surface
I Noda 143

Ice-core record of oceanic emissions of dimethylsulphide during the last climate cycle
M Legrand, C Fenist-Saigne, E S Saltzman, C Germain, N I Barkov & V N Petrov 144

Amelioration of subsoil acidity by application of a coal-derived calcium fulvate to the soil surface
H v H van der Watt, R D Barnard, I J Cronje, J Dekker, G J B Croft & M M van der Walt 146

Cryptomonad algae are evolutionary chimaeras of two phylogenetically distinct unicellular eukaryotes
S E Douglas, C A Murphy, D F Spencer & M W Gray N&V 148

Worldwide migration of amplified insecticide resistance genes in mosquitoes
M Raymond, A Callaghan, P Fort & N Pasteur N&V 151

Action potentials must admit calcium to evoke transmitter release
R M Mulkey & R S Zucker 153

Nature of the motor element in electrokinetic shape changes of cochlear outer hair cells
P Dallos, B N Evans & R Hallworth 155

Tyrosine phosphorylation and tyrosine kinase activity of the *trk* proto-oncogene product induced by NGF
D R Kaplan, D Martin-Zanca & L F Parada 158

A cellular protein that competes with SV40 T antigen for binding to the retinoblastoma gene product
S Huang, W-H Lee & E Y-H P Lee 160

Alternative temporal control systems for hypodermal cell differentiation in *Caenorhabditis elegans*
Z Liu & V Ambros 162

Function of DnaJ and DnaK as chaperones in origin-specific DNA binding by RepA
S Wickner, J Hoskins & K McKenney 165

Structural architecture of an outer membrane channel as determined by electron crystallography
B K Jap, P J Walian & K Gehring 167

A haemoprotein with kinase activity encoded by the oxygen sensor of *Rhizobium meliloti*
M A Gilles-Gonzalez, G S Ditta & D R Helinski 170

Single-site enzymatic cleavage of yeast genomic DNA mediated by triple helix formation
S A Strobel & P B Dervan 172

Meteoritic silicon carbide: pristine material from carbon stars (Erratum)
R S Lewis, S Amari & E Anders 174

N&V **See News and Views**

CLASSIFIED

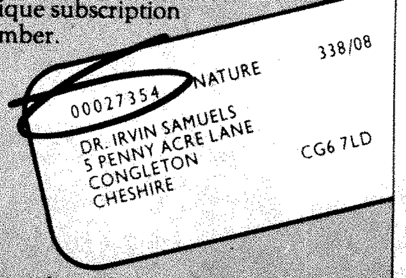
Professional appointments ■ Research posts ■ Studentships ■ Fellowships ■ Conferences ■ Courses ■ Seminars ■ Symposia Back pages

NEXT WEEK IN NATURE

Hubble's constant determined with aid of a lensed quasar ■ Revised view of greenhouse warming ■ Nitrate removal from water ■ A memory for bird song ■ Maize gets *Knotted* ■Transgenic superantigens

SUBSCRIPTION ENQUIRIES

Your mailing label contains your unique subscription number.



Please make a note of your number and quote it whenever you contact us about your subscription.

Annual Subscription Prices (including Annual Index)

UK		£136
Rest of Europe		£145
USA & Canada		US$350
Australia & NZ	Airspeed	£165
	Airmail	£215
India	Airspeed	£132
	Airmail	£215
Japan		¥95000
Rest of World	Surface	£149
	Airmail	£215

Orders (with payment) & subscription enquiries to:

USA & Canada
Nature
Subscription Dept
PO Box 1733
Riverton
NJ 08077-7333 USA

UK & Rest of World
Nature
Subscription Dept
Brunel Road, Basingstoke
Hants RG21 2XS, UK
Tel: 0256 29242

(The addresses of Nature's editorial offices are shown facing the first editorial page)

Japanese subscription enquiries to: Japan Publications Trading Company Ltd, 2-1 Sarugaku-cho 1-chome, Chiyoda-ku, Tokyo, Japan. Tel: (03) 3292 3755

Personal subscription rates: These are available in some countries to subscribers paying by personal cheque or credit card. Details from:

USA & Canada
Nature
65 Bleecker Street
New York
NY 10012, USA
Tel: (212) 477-9600

UK & Europe
Marketing Department
Nature
4 Little Essex Street
London WC2R 3LF, UK
Tel: 071-872 0104

Back issues: UK, £3.00; USA & Canada, US$15.00; Rest of World, £4.00 (surface), £5.00 (air)

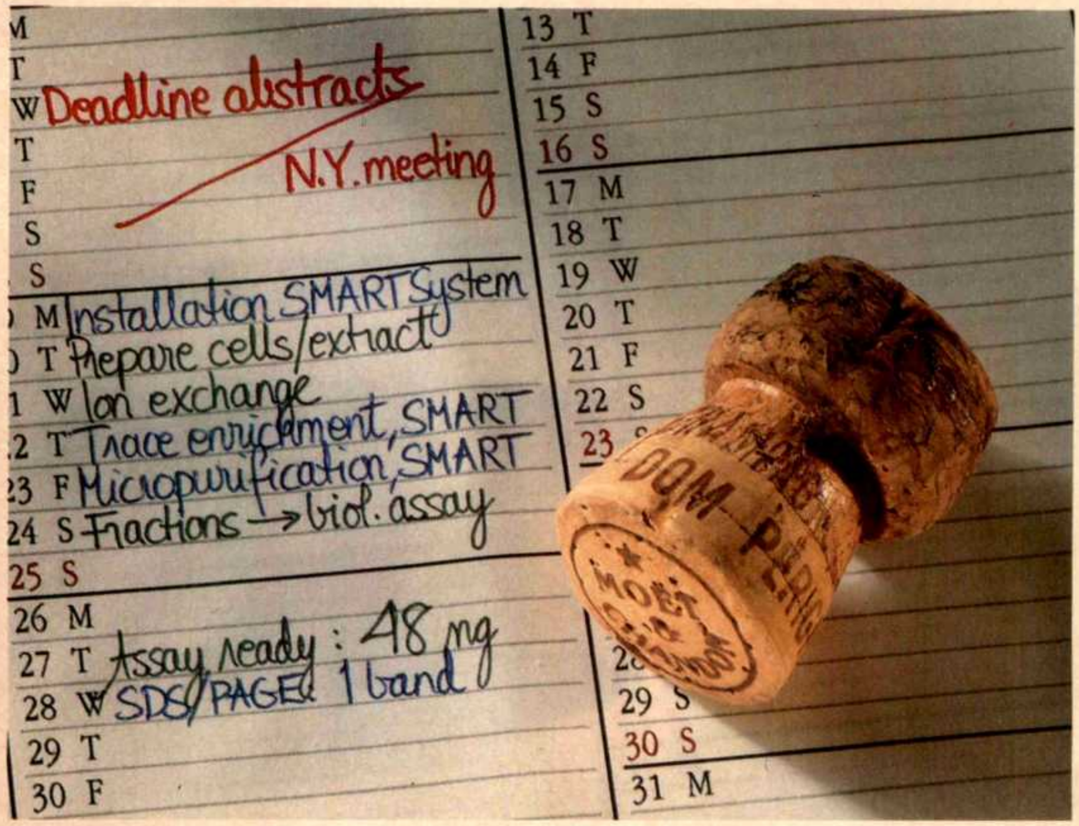
Binders
Single binders: UK, £5.25; USA $10.95; Canada $12.45; Rest of World, £7.75. Set of four: UK, £19.50; USA $39.95; Canada $45.95; Rest of World, £28.00

Annual indexes (1971-1989)
UK, £5.50 each; Rest of World £7.00; USA & Canada US$25.00

Nature First Issue Facsimile
UK £3.50; USA/Canada US$25.00; Rest of World £6.00

Nature in microform
Write to University Microfilms International, 300 North Zeeb Road, Ann Arbor, MI 48106, USA

PHARMACIA LKB BIOTECHNOLOG



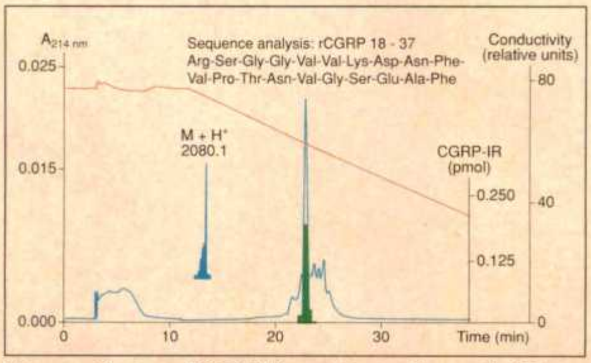
"My definition of success has expanded from non-existence to 48 ng."

November 28, 1990.
A day of celebration. We finally have more than enough of what it takes to be successful: 48 ng of pure concentrated peptide for further investigation by micro-analytical techniques. And we only had 50 ng to begin with!

Working with the state-of-the-art in micropurification, SMART™ System, was all that we needed.

With SMART System, we've learned to expect a maximum return on injection, e.g. 78% of ovalbumin at 1 ng. We have evidence of efficient sample concentration and trace enrichment, e.g. 50 ml to 50 µl in a single step. And we've exchanged buffers rapidly with better than 95% recovery.

With SMART System, we are also able choose the best separation technique for our sample. And when it comes to defining resolution, a single peak can be recovered in a 5 µl fraction!



Micropurification of CGRP-like activity in 50 ng with 95% activity, together with results from subsequent sequencing and mass spectrometry.

Our definition of success in micropurification has changed since we have gotten SMART System. Now we know that there ought to be enough left for everyone to join in on the celebration. Ask for the brochure.

SMART System, when you need to do a lot with a little.

Reader Service No.546

Pharmacia

Pharmacia LKB Biotechnology

We help you manage biomolecules

Head office Sweden Tel 46 (0)18 16 30 00 **Australia** Tel 61 (0)2 888 36 22 **Austria** Tel (0222) 68 66 25 0 **Belgium** Tel (02) 242 4660 **Brazil** Tel 011 288 9122 **Canada** Tel (514) 457 6661 **Denmark** Tel 42 26 52 00 **Federal Republic of Germany** Tel 49 (0)761 490 30 **Finland** Tel (90) 5021 077 **France** Tel 1-30 64 34 00 **Great Britain** Tel (0908) 661101 **Holland** Tel 03480 77911 **India** Tel (0812) 269631 **Italy** Tel 02 273221 **Japan** Tel 03-492-9481 **Norway** Tel (02) 54 90 95 **People's Republic of China** Tel 852 814 8421 Tel 861 256 5603 **Republic of Korea** Tel 02 5110801 **Spain** Tel 34 (9)3-675 44 11 **Sweden** Tel 08 623 85 00 **Switzerland** Tel (01) 821 18 16 **United States** Tel (201) 457 8000 **Eastern Europe** Tel 43 222 982 1607 **Far East** Tel 852 814 8421 **Middle East** Tel 30 (0)1 962-7396 **Other countries** Tel 46 (0)8 799 80 00 (90 06) 1149

Excellence in biotechnology

AT LAST! THIS SCIENTIST HAS FOUND
ELECTROPORATION CUVETTES
AT THE BEST PRICE
EASY TO USE
EACH CUVETTE COMES WITH A CAP
2 OR 4 MM CUVETTE WITH DISPOSABLE PIPETTE
IN NEW IMPROVED-PERFORMANCE ALLOY
A SAMPLE WITH 200µl CONE IS ALL YOU NEED TO RETRIEVE YOUR LIQUID

- Each **25-cuvettes** bag contains individually packaged disposable cuvettes. The whole set is **sterilized** by gamma irradiation.
- Fully compatible with the **"CELLJECT"*** Electroporation System and **others**.
- A very attractive price.

Reader Service No. 620

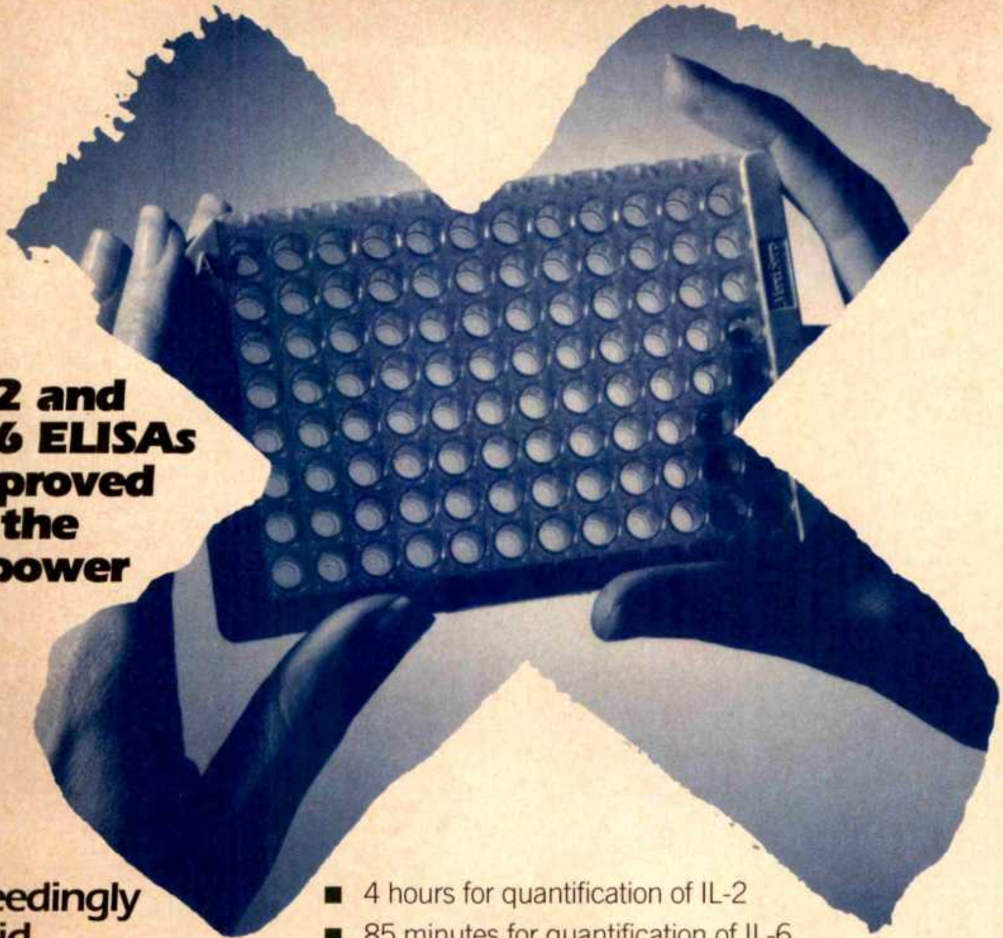
* "CELLJECT" is our high performant electroporation apparatus with its unique "Double pulse" system.

EUROGENTEC S.A.

Information and orders :
EUROGENTEC S.A.
Campus du Sart Tilman
Allée du 6 Août B6 - B 4000 Liège - BELGIQUE
Tel : ++ 32 41 56 33 79 - Fax ++ 32 41 56 29 68

Name :
Title : Dept. :
Institution :
Address :
Postal Code: Country :
Phone number: Fax number:

INNOVATION



IL-2 and IL-6 ELISAs improved to the X power

Xceedingly rapid
- 4 hours for quantification of IL-2
- 85 minutes for quantification of IL-6

Xtraordinarily flexible
- Run assays sized to your needs
  - —96 individual, break-away wells
  - —stable reagents; enough for four separate assays/kit

Xquisitely accurate
- High sensitivity, linear standard curves
  - —150-4050 pg/mL for IL-2
  - —160-2500 pg/mL for IL-6
- Standardized to the WHO standards

Xceptionally easy to use
- Rapid, easy to use ELISA format
- Includes everything you need: precoated microtiter plate, standard, buffers, and detection system

Xtra value
- Cost-effective—use only what you need—when you need it
- Extended shelf-life—run an assay now, then another months later

New and improved

HUMAN INTERLEUKIN-2 ELISA KIT
INTERTEST-2X™

HUMAN INTERLEUKIN-6 ELISA KIT
INTERTEST-6X™

genzyme

Genzyme Corporation
One Kendall Square
Cambridge, MA 02139 USA

©1991, Genzyme Corporation
INT 001 February 1991

For more information or to place an order, call 1-800-332-1042 or FAX 617-252-7700. Outside the USA, contact your Genzyme Distributor or call 617-252-7500.

For Research Use Only. Not For Use in Diagnostic Procedures.

Reader Service No. 622

A new start with STATISTICA!

FOR IBM USERS

CSS/3 Complete System - over 1000 quality graphs with on-screen customisation, fully integrated with all procedures - largest stats selection in one package - unlimited file-size.

QUICK/CSS All basic modules of CSS/3 + data management. Full graphics with on-screen customisation CSS GRAPHICS. Largest graphics selection in one package as in CSS/3, on-screen drawing, insetting, multigraphs, 30 types rotating 3D graphs and much more.

STATISTICA Fully integrated, combining CSS/3, CSS GRAPHICS and MEGAFILE MANAGER. Extremely comprehensive all-in-one data analysis package.

MEGAFILE MANAGER Most comprehensive data base system - Up to 32000 fields per record, unlimited records, data entry, verification, restructuring, programming.

FOR MACINTOSH USERS

STATISTICA/MAC The CSS-compatible data analysis and graphics system with 3D rotation and on-screen drawing unlimited size of files.

QUICK CSS/MAC All basic statistical modules. Full quality graphs.

QFAST Computerised testing system - menu driven programme - stores results - prints reports. For IBM 256K and Macintosh.

LARGE DISCOUNTS
FOR
MULTIPLE ORDERS!
WE ACCEPT CREDIT CARDS

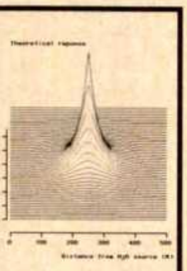
For details please phone
0462 482 822 Fax 0462 482 855
or write to:
StatSoft UK Icknield House, Eastcheap, Letchworth, Herts SG6 3DA.

StatSoft UK
Sole UK Distributors

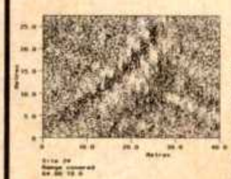
Reader Service No.7

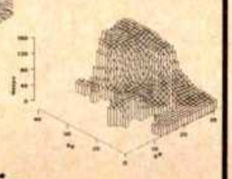
EUROPEAN DISTRIBUTORS OF STATISTICAL SOFTWARE

3D Surface & Mapping Software for your Mac



- Five types of 3D Surface plot/3D Graph.
- Contour, dot-density, point location, & shadowgram maps.
- Accepts gridded data & scattered XYZ values as input.
- Extensive grid/grid-to-grid data manipulation.
- Area & volume calculations, trend surfaces, filtering.
- World maps to 12+ different projections showing coastlines etc. (data supplied). Add your own line, polygon & point features.





Version 2 available
March 1991.
Write or phone for
a descriptive leaflet.

WTC Scientific

152 Buxton Road, Macclesfield
Cheshire SK10 1NG, UK.
Phone 0625 420210 Reader Service No.8

REFERENCE REAGENTS FOR HUMAN CYTOKINES

The Biological Response Modifiers Program (NCI), the Division of Microbiology and Infectious Diseases (NIAID), and the National Institute for Biological Standards and Control (United Kingdom) have made available reference reagents for human cytokines. The reagents are available in small amounts (approx. 1μg/sample) for use in the calibration of *in vitro* assays and in-house standards only, and are not to be used for experimental purposes.

NEW REFERENCE REAGENTS:

IL-8
TNF-ß

OTHER REFERENCE REAGENTS AVAILABLE:

IFN-α
IFN-ß
IFN-γ
IL-1α
IL-1ß
IL-2
IL-3
IL-4
IL-6
G-CSF
GM-CSF
M-CSF
TGF-ß1
TNF-α

To Obtain These Reagents, Contact:

Dr. Craig W. Reynolds
Biological Response
Modifiers Program
NCI-FCRDC
Building 1052, Room 253
Frederick, MD 21702-1013

Shipments will be made collect express. Please allow 3 to 4 weeks for delivery.

NBSB
NATIONAL INSTITUTE FOR
BIOLOGICAL STANDARDS AND CONTROL

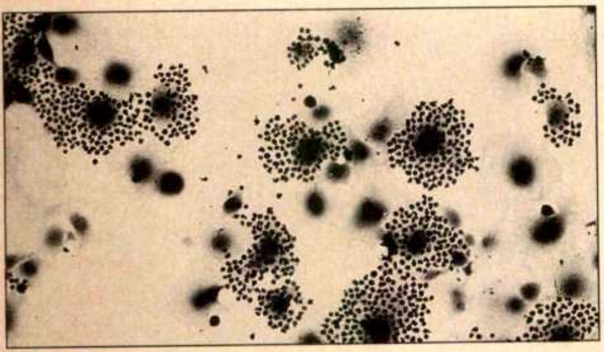
National Cancer Institute —
Frederick Cancer Research Facility

Reader Service No.42

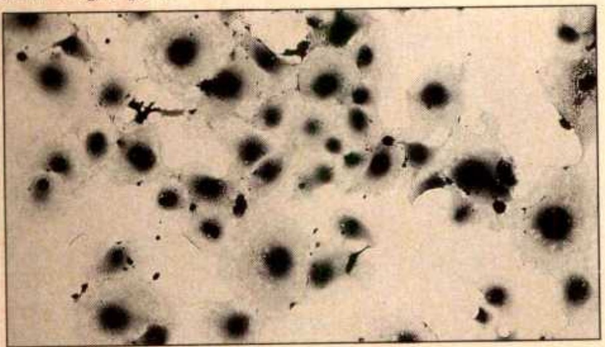
Endothelial Adhesion Molecules

British Bio-technology offers a range of products designed to aid research into the functions and roles played by the novel adhesion molecules:

ICAM-1
ELAM-1
VCAM
PECAM



Control. Rosettes of U937 cells binding to COS cells expressing the ELAM Designer gene.



Inhibition of U937 adhesion to COS cells expressing the ELAM gene by pre-incubation of the COS cells with anti-ELAM antibody.

MONOCLONAL ANTIBODIES High titre, specific monoclonals purified from hybridoma supernatants, suitable for immunocytochemistry and adhesion blockade.

DESIGNER GENES Full Length cDNA clones supplied in a tested expression vector for easy expression of proteins.

DESIGNER PROBE COCKTAILS A cocktail of 6 region specific, antisense 30 mers ideally suited for the detection of mRNA or DNA in techniques such as *in situ* hybridisation or Northern/Southern blotting.

Call Direct (0865) 781045

British Bio-technology Limited

4-10 The Quadrant, Barton Lane,
Abingdon, Oxon OX14 3YS.
Tel: (0235) 529449 Fax: (0235) 533420
Telex: 838083 BIOTEC G

Reader Service No.37

NATURE EDITORIAL OFFICES

LONDON: 4 Little Essex Street, WC2R 3LF
Telephone: (071) 836 6633 Fax: (071) 240 6930

Editor: John Maddox

Managing Editor: Nuala Moran

Editorial Staff: Peter Aldhous, Philip Ball, Maxine Clarke, Christopher Coath, Rosalind Cotter, Alexander Gann, Laura Garwin, Henry Gee, Rory Howlett, Tim Lincoln, Philippa Lloyd, Anne Martin, Lindsay Matthews, Geoffrey North, Roland Pease, Miranda Robertson, Teresa Scranney, Peter Tallack, Graham Tebb, Richard Vile and Charles Wenz.

Art & Design: Nicola Barker, Jacqueline Dowdall and Stephen Sullivan

Administration: Mary Sheehan, Lucy Bending, Tamara Boake, Joanna Casale, Tania Coad, Jacqueline Hood, Barbara Izdebska, Ruth Lefèvre, Johanne Peters, Helen Slack, Anne Vericel and Rosalind White

WASHINGTON: 1137 National Press Building, DC 20045
Telephone: (202) 737 2355 Fax: (202) 628 1609

Deputy Editor: Barbara J Culliton

Editorial: Christopher Anderson (News), Kevin Davies (Biology), Diane Gershon (Product Review and News), David Lindley (Associate Editor) and Robert Pool (News Editor)

Administration: Penelope Walker, Kathryn Bielenberg and Laura Bellows-Ancasi

MUNICH: Sandstrasse 41, 8000 München 2
Telephone: (089) 527036 Fax: (089) 523 2222

Munich Correspondent: Steven Dickman

TOKYO: Shin-Mitsuke Building (4F), 3–6 Ichigaya Tamachi, Shinjuku-ku, Tokyo 162
Telephone: 03 3267 8751 Fax: 03 3267 8746
Tokyo Correspondent: David Swinbanks
Promotion Manager: Koichi Nakamura
Japan Advertisement Manager: Phillip Hamill
Administration: Rinoko Asami

Managing Director: Ray Barker

Editorial Director: Mary Waltham

Marketing Manager: Samantha Dawes

International Advertisement Manager: Fiona Anderson

International Sales Manager: Julie Bryant

Sales Executives: Angela Burry, Emma Hipwell and Daryl Rayner

Classified Advertisement Manager: Julie Skeet

NEW YORK OFFICE: 65 Bleecker Street, New York, NY 10012
Telephone: (212) 477 9600 Fax: (212) 505 1364
President: Andy Sutherland
Vice Presidents: Marion Delaney and James A. Skowrenski
American Advertising Manager: Ralph Frakes
Classified Advertising Manager: Marianne Ettisch
Advertising Consultant: Henry Dale

CANADA: Display & Classified: Peter Drake, 17 Pine Crescent, Toronto, Ontario M4E 1L1. Telephone: (416) 690 2423

DISPLAY ADVERTISING REPRESENTATIVES

New York: Nature Office
Chicago: Didier & Broderick, Inc. (708) 498 4520
West Coast and Southwest: Lynne Stickrod (415) 781-3801
East Coast: Glenn Melvin (212) 477 9608
France: Clare Newell Tel. (1) 48 87 24 43/Fax (1) 48 87 46 71
Switzerland: Verena Loewenthal, Interpress, CH-8103 Unterengstringen, Zurich (01) 750 5511/Fax (01) 750 2210
Israel: Daniel Zimet, Zimet Advertising & Publishing (03) 261653/Fax (03) 253391
Japan: Philip Hamill (03) 267 8751
Netherlands and Belgium: Adele Struyck, Astra, Netherlands (03431) 1702
Scandinavia: Andrew Karnig, Andrew Karnig & Associates, Sweden (08) 4400 05
Germany and Austria: Sabine Fürst Tel. (089) 524081/Fax (089) 523222

Nature® ISSN 0028-0836
Registered as a newspaper at the British Post Office
© 1991 Macmillan Magazines Ltd
Vol. 350 No. 6314 14 March 1991

NATURE VOL 350 nature 14 MARCH 1991

Independence of forensic science

The third appeal of six people against life sentences imposed 15 years ago raises sombre questions about the management of forensic services, not to mention the quality of British justice.

THE application of scientific techniques to the detection of crime and the conviction of criminals has a long and even a spectacular history, with the result that the achievements of forensic science have spawned a whole genre of airport fiction. But real life, usually less glamorous, is full of pitfalls for the unwary. So much is plain from the third re-hearing now under way by the British Appeal Court of the evidence for the conviction in 1975 of six Irishmen for having killed 24 people (and injured more than 120) by exploding a bomb in a crowded drinking-house in Birmingham. The 'Birmingham six', as they are known, were convicted and imprisoned for life on evidence of two kinds: purported confessions to the police who had arrested them and evidence (accepted by the trial court as valid) that two of them had been found on forensic investigation to have traces of explosives on their right hands.

What now emerges is that the forensic evidence presented at the original trial was flawed, and has for practical purposes been withdrawn. A chemical procedure for identifying nitroglycerine has now been found to have been inadequately controlled: materials other than nitroglycerine (including soap) can yield positive results. Results of an investigation by gas chromatography, potentially a more sensitive technique, were acknowledged in evidence last week to be similarly capable of confusion by other materials (and, even so, were positive for the *left* hand of only one of the two supposedly contaminated defendants). But forensic science has salvaged some of its reputation by producing evidence (still formally to be accepted by the Appeal Court) that the disputed confessions produced by the police consisted of writings (by the interrogating officers) that were neither contemporaneous nor consecutive.

Two important issues stem from these alarming disclosures, of which the chief is more than a mere embarrassment to the British government and judicial system. If, as seems probable, the Birmingham six are now released after 16 years in jail, this will be yet another case in which zealous and overzealous policemen have been prepared to concoct evidence in the pursuit of a conviction. That many of these cases have involved supposed terrorism by the Irish Republican Army has not, to say the least of it, simplified the management of Ulster's problems.

The forensic issue is, or should be, more tractable. In Britain, the Forensic Science Service has traditionally been run by the Home Office as a common service for Britain's police forces. But from 1 April, it will become an executive agency — expected to pay its way but not to make a profit. The forensic service, which has six laboratories south of the Scottish border, will take business from all potential customers: police forces, but also defendants before the courts. The danger is that the service will be yet more dependent on the wishes of its customers, includinge policemen out for a conviction.

That is why a still more radical change is required. It was claimed (by counsel for the appellants) at last week's hearing that evidence that the original test for nitroglycerine was not reproducible had been withheld from the trial court — and from defence lawyers. It is only fair (to the forensic service 15 years ago) to acknowledge that the court might have learned the truth if the defence lawyers had asked the right questions (and only fair to them to acknowledge that lawyers are rarely trained in the niceties of laboratory technique).

Yet there can be few occasions when the results of forensic investigations are as clear-cut as the airport literature would have the rest of us believe, let alone when lawyers appreciate what the uncertainties of forensic investigations mean. So much has been well illustrated by the arguments over the unreflective use of DNA fingerprinting by some US courts (see *Nature* **339**, 501; 1989). The only durable solution is that forensic services everywhere should become the servants of the courts at which their evidence is presented, not of those who commission their investigations.

In British circumstances, the simple solution is that they should be a child of the Crown Prosecution Service, which has (among other things) responsibility for investigating the quality of evidence against accused persons. That is the solution advocated by Lord Scarman, Britain's most distinguished ex-judge. But why not go further, and make them dependent on the courts as such, as are the bailiffs who go about collecting debts? The snag, for the government, would be that the prospect that the forensic services would be self-financing might then melt away, but when the reputation of the judicial system has been undermined by faulty evidence, that hardly seems a substantial consideration. □

Technology untied?

Plans to liberalize British telecommunications would be more commendable if they were more radical.

SINCE soon after the time of Alexander Graham Bell, governments have asserted a right to regulate the uses made of his invention and its successors, and for two kinds of reasons. First, as in the pre-*glasnost* Soviet Union, telephones provide people with potentially subversive opportunities for communication, which governments seek to prevent either by not providing a workable service or by listening to the conversations that telephones generate. But even elsewhere, telecommunications services are not free. Because telephone networks are natural physical monopolies, public regulation of even private enterprises is unavoidable. It is not merely that unregulated communications networks might exploit their obligatory customers financially, but that they might discriminate among them on political or other such grounds. During last year's elections in Eastern Europe, for example, opposition parties complained loudly that the state providers of communications gave them too little access to the inadequate facilities that there were.

Given all that, the British government deserves a little credit for having decided, last week, that there should now be a further liberalization of the regulatory framework of British telecommunications. (During the same week, it also decided that all airlines can in future apply for the right to land at London's principal airport, which is a further sign of liberal inclinations — see *Nature* **349**, 638; 21 February 1991). Yet what the government now proposes is the least of what it should have done a decade ago, when 51 per cent of the publicly owned telecommunications monopoly (called British Telecom) was sold to the public. And the snail's pace of regulatory change promises to lag further behind the pace of technical change — which, ironically, the government seeks further to accelerate.

In the new regime, as in the old, operators of telecommunications services will have to be licensed, which is proper, while the scope of the services they provide and the prices at which they sell them will continue to be overseen by a regulatory agency, called Oftel. Ten years ago, the government licensed British Telecom to provide a comprehensive telecommunications service (nationally as well as overseas) and encouraged a second (called Mercury) to build a competing network. But the government was also then grappling with (even cheerleading) the prospect that the opportunities in cable television would quickly thread the country with an independent broad-band telecommunications network. (The prospect might have become reality had the government not fortuitously moved the financial goalposts.) Meanwhile, all concerned have evidently been astonished by the pace of development of mobile networks based on UHF radio.

What is now proposed is a modestly sensible loosening of this framework. Newcomers to the communications business will in principle be licensed (but not immediately). Meanwhile, operators of mobile communications systems and of cable networks will be allowed to use their facilities to provide fixed-point, local and even trunk communications. Private organizations wishing to set up communications systems by means of Earth satellites will be allowed to do so, provided that their channels are not connected to a fixed network at either end. Both British Telecom and Mercury will have to allow others access to their networks on terms to be negotiated, and will also be allowed to continue subsidizing their domestic services and capital investments by making uneconomically high international charges (which are nevertheless to be reduced by 10 per cent). At least for seven years, perhaps for ten, British Telecom will not be allowed to use its network for distributing television signals.

The last of these restrictions points directly to the technical anomaly now being created. With broad-band communications just around the corner, it would make technical sense that there should be a single broad-band network based on optical-fibre cables — and one set of holes in urban streets. But would not that be a recipe for domination of the market by its most likely provider, British Telecom? Not if the regulatory framework required that provider to function as a common carrier, charging transparent prices for access to the network and having itself no commercial interest in the uses made of the network — voice communications, data transmission or even entertainment. The British government's difficulty is that, even if it is not influenced by the 49 per cent of the company it has retained, it has a kind of moral commitment to those who own the rest of the stock to ensure that the company is not emasculated commercially. But the perpetually managed profitability of British Telecom is hardly a more laudable objective than those that embarrassed successive governments when British Telecom was a nationalized industry. □

Women without men

A row about the insemination of unmarried women scorns human nature and undervalues human ingenuity.

WITH the Gulf War over for the time being, the British tabloid press and even some members of the House of Commons (MPs) are making a fuss about the revelation that some women (there are at least two) have persuaded a fertility clinic to enable them to become pregnant with sperm from a donor-bank. "Virgin birth" features in most headlines, but there is also a drum-beat of thundering about the sanctity of the nuclear family, and yet another outburst of anxiety about the new human embryology (misplaced because artificial insemination by donor, or AID, is old hat).

What seems to have been overlooked is that many unmarried women have traditionally sought to bear children, and to bring them up on their own, without the assistance of fertility banks. Nobody could possibly tell what proportion of the large number of unmarried mothers in Britain are in this category, but it would not be negligible. Two parents are better than one, and not simply for the obvious prudential reasons (one might die). But these days it takes a brave newspaper or MP to say that a woman without a mate must not become pregnant. And who is to say that mating with a passing aquaintance is preferable to a fertility bank? Is it possible that the indignation so suddenly aroused stems from the British government's plan to insist that absent fathers make maintenance payments on behalf of their children? Will the fertility banks be responsible? □

Human Frontier effort gets promise from NIH

■ Institutes offer in-kind contributions
■ Psychological and political benefits

Tokyo & Washington

A LETTER from a senior official at the US National Institutes of Health (NIH) indicates that NIH have decided to contribute to the Human Frontier Science Program (HFSP), the ground-breaking Japanese effort to support international research on the brain and molecular biology through a foundation based in Strasbourg, France.

Although the US contribution will be relatively small and will be confined to support of researchers working in the United States, the NIH decision comes at a very opportune time for the Human Frontier programme — it is nearing the end of a three-year trial period in which nearly all its financial support has been provided by Japan, and it is now entering a critical phase of reassessment. Furthermore, the promise of an NIH contribution marks a remarkable reversal in the US attitude towards the programme, which was greeted with suspicion by the United States when it was first announced by Japan's Ministry of International Trade and Industry (MITI) in 1986.

The NIH decision was announced by Edward Rall, its deputy director of intramural research, in a letter dated 26 February to Sir James Gowans, the British secretary-general of the programme in Strasbourg. On Monday, however, there seemed to be some confusion in NIH as to just how far the decision-making process had gone. Jerome Green, who is NIH's representative on the Frontier programme's board of trustees, said there had been no final decision on making contributions in kind, "letters and rumours to the contrary".

Rall was out of town and not available for comment, but his letter clearly indicates that the NIH policy is set. The letter states that NIH will make an 'in kind' contribution for HFSP fellowships as follows: "Any fellow who is duly selected by the Fellowship Panel and approved by the Council of Scientists, and who plans to work at any laboratory in the National Institutes of Health, or the Alcohol, Drug Abuse and Mental Health Administration, will be supported by the National Institutes of Health in accordance with the same stipend level as the HFSP would provide." A notation at the bottom of the letter indicates that copies were sent to several science policy officials in Washington, although not to Green.

Such in-kind funding from the NIH will probably not cost the agency a great deal of money. In fiscal year 1990, only a handful of scientists were awarded HFSP fellowships to work at NIH laboratories, and with a similar number of awards expected this year, the size of the US contribution will amount to only a few hundred thousand dollars at most.

That is just a small percentage of the total cost. In fiscal year 1990, the Frontier programme awarded 29 grants of up to $400,000 a year to international teams, 80 postdoctoral fellowships worth $40,000 a year and ten workshop grants of $80,000 each. Nearly $20 million, or more than 75 per cent of the $26 million budget for the programme in fiscal year 1990, was provided by MITI and Japan's Science and Technology Agency (STA). The remainder was made up from $4.2 million carried over from fiscal year 1989, and relatively small contributions from four of the other six summit nations that belong to the HFSP: France ($1.24 million), Italy ($0.24 million), Canada ($0.20 million) and Germany ($0.12 million). (The United Kingdom and the United States have made no cash contributions.)

But despite the relatively small size of the forthcoming NIH contribution, psychologically and politically it is very important.

Japanese backers of HFSP have been seriously concerned that the programme might run into funding difficulties in Japan if other summit nations, particularly the United States, did not contribute more. At the end of fiscal year 1991 (31 March 1992), the programme comes to the end of its three-year trial period, and the Japanese ministry of finance must decide if — and on what scale — to continue. Professor Akiyoshi Wada, one of the two Japanese members of the HFSP council of scientists, said last week that the lack of contributions from other countries was threatening the future of the programme.

The failure of the programme would be a great loss not only for scientific reasons, he said. Many science and government officials in the summit nations think highly of the programme. For instance, Philip Schambra, director of the Fogerty International Center at NIH, says that HFSP fulfils an important function in encouraging international cooperation in biological research and should be expanded. But Wada points out that the programme has meant even more than that to Japan. Although few people in the West realize it, he said, the programme has had tremendous beneficial effects in Japan by exposing some of the domestic bureaucratic barriers facing the internationalization of science. He likens Frontiers to the coming of the US 'black ships' led by Commodore Matthew C. Perry in the last century, which opened up Japan to the West after over two centuries of isolation.

So Wada was relieved to hear the news that NIH and Switzerland (see below) will contribute to HFSP, and says he is now more optimistic about the future.

In January, the HFSP board of trustees began the process of reassessing the programme. That process will be concluded in June — just in time for MITI and STA to make budget applications for fiscal year 1992. The board is expected to recommend a further two-year trial period rather than giving its full-blooded support because it is too early to assess the scientific achievements of the programme. Wada and Japanese government officials in charge of the programme hope that the votes of confidence from NIH and the Swiss will be enough to convince the Ministry of Finance not only that HFSP is worth continuing but also that it deserves increased support.

David Swinbanks & Robert Pool

Swiss sign up also

SWITZERLAND has been officially accepted into Japan's Human Frontier Science Program (HFSP), becoming the first nation to join the programme that was not one of the seven Summit nations that were the original members.

The newest member will contribute $400,000 a year, making it the third largest contributor after Japan and France. The acceptance of Switzerland into HFSP may stimulate other non-Summit nations to sign up for the programme, which supports international research on the brain and molecular biology.

Switzerland, Sweden and Australia have long expressed an interest in joining the Frontiers programme, but an initial offer from these countries last year was rebuffed by the HFSP board of trustees. The deal fell through partly because the board considered the amount offered (about $150,000 a year) to be too small. A second problem was that the Japanese officials negotiating the deal had offered preferred treatment to nations willing to make contributions, says Katherine Bick, who represented the United States on the board of trustees at that time. She says the board told the Japanese government that the grants would have to be made strictly on merit. And so the matter went back to the negotiating table (see *Nature* **345**, 9; 1990).

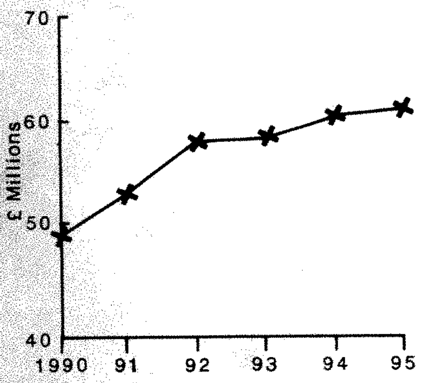
Japanese government officials say they have not heard from Sweden or Australia recently and the "ball is now in their court", but Switzerland is now a fully fledged member of the programme. Two Swiss have been nominated to the board of trustees, and a Swiss scientist will join the council of scientists when it meets in Strasbourg next week. **D.S. & R.P.**

PARTICLE PHYSICS

Does Europe need the LHC?

London

Sir David Phillips, chairman of the Advisory Board for the Research Councils (ABRC), last week angered British physicists by questioning in public the need for a separate European programme in particle physics. His comments brought out into the open a long-running debate about whether the world needs two science projects costing thousands of millions of dollars each to address essentially the same question.



Phillips's headache — the planned UK subscription to CERN, 1990–95. Source: SERC.

Speaking to the House of Lords Science and Technology Committee, Phillips said that particle physics should be coordinated at a global level, but the "competitive spirit" of rival groups of physicists has won through. Science advisers in France and Germany, as well as in Britain, are concerned about the level of planned expenditure at CERN, the European particle physics laboratory in Geneva, Phillips said. The SFr 1730 million (about £680 million) cost of building a new accelerator, the Large Hadron Collider (LHC), is particularly worrying, he said, because the plan coincides with a similar US initiative — the $8,300-million Superconducting Super Collider (SSC) project.

The British subscription to CERN, at more than £50 million a year, is the largest single component of the UK science budget. So, as he is chairman of the ABRC, which advises the Education and Science Secretary on the size and distribution of the science budget, Phillips' concern about future increases in the CERN subscription is understandable. But his comments come at a sensitive time: the CERN member nations must next year decide whether or not to go ahead with the LHC. Carlo Rubbia, director-general of CERN, last week declined to comment on the criticisms, preferring to let British physicists rally to CERN's cause.

CERN's supporters argue that if there is an undesirable overlap between the LHC and SSC projects, the fault lies with the United States. Sir William Mitchell, former chairman of the UK Science and Engineering Research Council (SERC), says that CERN and the British government were pushing hard for a world strategy in particle physics in the late 1980s. But "it takes two to tango", he says — the United States was determined that the next stage in its particle physics programme would be a US-dominated project. Sandy Donnachie, chairman of SERC's Nuclear Physics Board, which handles the UK CERN subscription, says the US attitude was: "we must regain the initiative from the Europeans". Nevertheless, Roy Schwitters, director of the SSC laboratory, responds that European physicists will be involved in experiments on the SSC, and are represented in his laboratory's peer review committees.

Mitchell, who is now president of CERN's governing council, believes the LHC project will not increase CERN's budget dramatically, as the new collider can be built around CERN's current centrepiece, the Large Electron–Positron collider, using some of the same equipment. Donnachie agrees: "if Europe were to buy into the SSC, it would finish up costing us more than the LHC."

Chris Llewellyn Smith, from the University of Oxford, says that competition between rival groups can be a spur to better science. He expects the LHC to be completed before the SSC, but says the LHC's greater versatility is in any case a powerful argument against simply pinning European hopes on a contribution to the SSC.

The primary function of both the SSC and the LHC is to collide two beams of protons. Proton collisions may yield the much sought after Higgs boson, predicted by current ideas in theoretical physics. The SSC will operate at a higher energy, but Llewellyn Smith says that greater intensity of the LHC proton beams should compensate for this, in part.

The LHC will also be able to operate as an electron–proton collider, providing the next step beyond the German HERA machine, where experiments start later this year. A third role is also planned, using the LHC to collide heavy ions. By forcing heavy nuclei together, physicists may be able to duplicate the 'soup of quarks' that must have existed early in the evolution of the Universe, Llewellyn Smith says. A separate $300-million US machine is planned to conduct similar experiments (but at a fraction of the energy the LHC will deliver) at the Brookhaven National Laboratory in New York State.

Peter Aldhous

OIL-WELL FIRES

Smog alert in Kuwait City

London

By concentrating on the large-scale environmental impact of the hundreds of oil-well fires now blazing in Kuwait (see *Nature* **350**, 11; 7 March 1991), scientists and the media may have neglected a more immediate problem — the threat to public health in Kuwait posed by toxic fumes released into the atmosphere.

At present, plumes of smoke and fumes are rising rapidly into the atmosphere, so dangerous concentrations of toxic chemicals are not building up at ground level in populated areas. But David Shillito, from the consultant chemical engineers Cremer and Warner, fears that Kuwait City will face a serious air pollution problem in the coming months, as seasonal winds begin to blow. When the wind speed hovers around 12 metres per second, Shillito says, the smoke plumes will be "blown over", but will not disperse rapidly.

In June and early July, a prevailing wind known locally as the Shamal blows over the northern Kuwaiti oil fields towards the capital. Shillito, who worked in the Persian Gulf for many years, says that the air quality in Kuwait City may then be as bad as the infamous London smogs of the 1950s, for periods of 2–3 days at a time. Oil-fire smoke contains a cocktail of noxious chemicals, from sulphur dioxide and other simple respiratory irritants, to carcinogens such as benzene and polyaromatic hydrocarbons.

Keith Browning, director of research at the UK Meteorological Office, says that his group has not been asked to extend its modelling of the environmental effects of the fires to assess in detail the likelihood of local air pollution events, but says that this "must represent a threat". Browning's group has considered the formation of ground-level ozone, by the action of sunlight on nitrogen oxides. Near the source of each fire, little light will penetrate the black smoke, but Browning says intermittent photochemical smogs are possible where the plumes fall back to the ground.

The Kuwaiti health ministry has asked the Geneva-based World Health Organization (WHO) for help in the aftermath of the war, and the air pollution threat is a top priority. WHO expects to announce this week that it is sending a team of clinical experts to Kuwait.

Another WHO concern is the serious risk of disease epidemics in Iraq, where allied bombing has caused severe disruption to water supplies and sanitation.

Peter Aldhous

BIOTECHNOLOGY PATENTS

Amgen scores a knockout

Washington

DESPITE expectations to the contrary, Amgen Inc. has emerged as the outright winner in its bitter dispute with Genetics Institute over US patent rights to erythropoietin (EPO). The US Court of Appeals for the Federal Circuit ruled that key claims in Amgen's patent were valid and enforceable and declared Genetics Institute's patent invalid.

The decision guarantees a market monopoly for Amgen. As a result, Genetics Institute will be unable to sell its version of EPO in the United States through its licensee Chugai Pharmaceutical Co. Ltd. EPO, which stimulates the growth of red blood cells, is proving to be a valuable therapy for patients on kidney dialysis, who often develop anaemia. AIDS patients with anaemia may also benefit from EPO.

News of the court decision took Wall Street by surprise and sent the company's shares up 12 per cent to close at $113. Just three weeks before the court's EPO decision, Amgen won US Food and Drug Administration (FDA) approval to market granulocyte-colony stimulating factor (G-CSF), another biotechnology drug. The colony stimulating factor enhances white blood cell production and is expected to find wide use among cancer patients and others who are suffering from immune deficiency.

Gordon Binder, Amgen's chairman and chief executive officer, stated that the court decision in the EPO case "vindicates our position completely". He believes that the ruling will encourage original research and development by reassuring the pioneers within the biotechnology industry that they can expect adequate patent protection for their inventions. Gabriel Schmergel, president and chief executive officer of Genetics Institute said "we are very disappointed with the decision" but would not indicate whether the company will take any further action.

The protracted patent dispute spanned three-and-a-half years and concerns conflicting US patent claims to EPO, a natural protein that is made in the kidney. In June 1989, the FDA approved Epogen, Amgen's genetically engineered version of EPO, for the treatment of anaemia in patients with chronic kidney failure. Estimated US sales of Epogen for 1992 are expected to top $400 million. Along with marketing approval, FDA awarded Epogen "orphan drug" status under an act that provides companies with incentives, including a guaranteed seven-year market monopoly, to develop drugs to treat rare diseases.

At the time, both Amgen and Genetics Institute held EPO patents. Amgen won its patent for discovery of the gene that produces EPO, whereas the Genetics Institute patent was awarded for isolation of the EPO molecule from human urine, as well as for preparation of a recombinant version of the protein. A court in Massachusetts, which heard the patent case in 1989, validated the central claims in both Amgen's and Genetics Institute's patents but declared that they were mutually infringing (see *Nature* **342**, 846; 1989). Observers expected that the appeals court in Washington, DC, would uphold the Massachusetts ruling and that the continued stalemate would force the companies to cross-license.

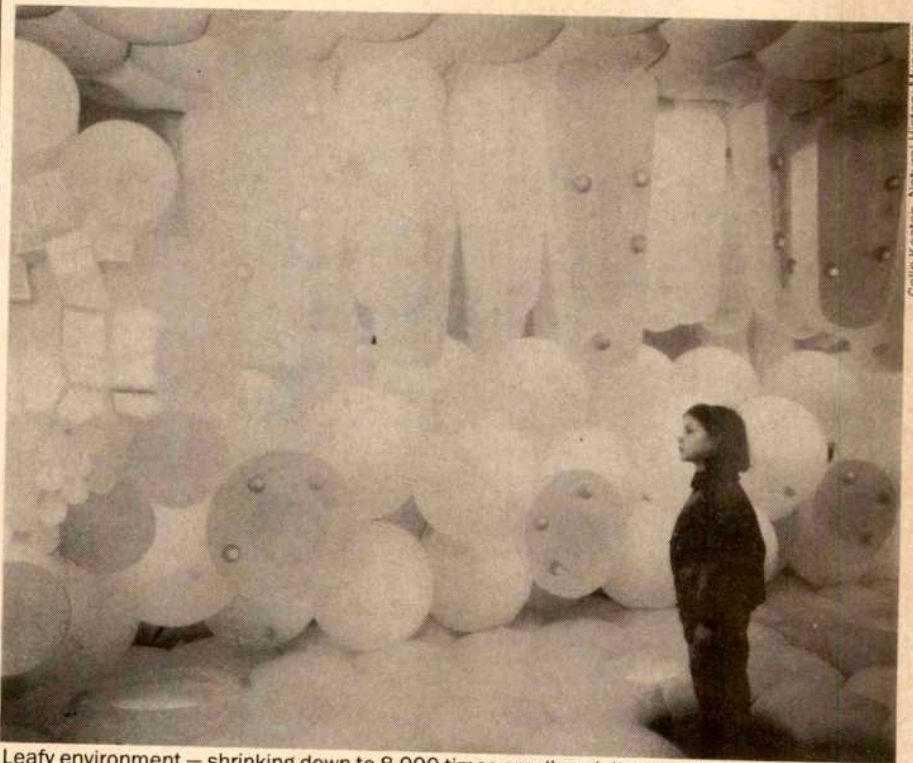
Although the federal appeals court affirmed Amgen's patent as valid and enforceable, Genetics Institute was dealt a severe and unexpected blow when the court struck down its patent claims to EPO. The court ruled that Genetics Institute and its licensee, Chugai, had failed to show that they had purified EPO, whether recombinant or urinary-derived, with the claimed specific activity using the methods disclosed in the patent. Genetics Institute had argued that its claim to EPO was stronger because Edward F. Fritsch of Genetics Institute first conceived the strategy to identify and isolate the EPO gene back in 1981, even though Fu-Kuen Lin of Amgen was first to accomplish this goal in September 1983, followed by Fritsch eight months later. The appeals court determined, however, that Fritsch "had no more of a conception in 1983 than he did in 1981 because he did not know the sequence of the gene encoding EPO."

Peter Drake, stock analyst with Vector Securities, paid tribute to Amgen's management team, who he said had the long-term vision, energy and commitment to defend its patent position "in the face of significant pressure from Wall Street and other circles to cross-license and put this [the patent dispute] behind them". Amgen is now in the enviable position of having "two of the crown jewels in the biotechnology industry", says Drake, who estimates that the company will have $1,000 million in sales by 1993.

Diane Gershon

NATURAL HISTORY MUSEUM

Innovative ecology exhibits



Colin Keates, Natural History Museum

Leafy environment — shrinking down to 8,000 times smaller, visitors to the ecology exhibition can take a tour around the 'inside' of a leaf.

A TOWERING glass greenhouse inside the Natural History Museum in London sets the scene for an impressive yet controversial permanent exhibition on ecology that opened on 7 March. It is the first of five new exhibitions scheduled to open over the next few years and sponsored by the museum's independent Development Trust Appeal. The exhibition marks an important shift away from government funding, but the in-house team largely responsible for the exhibition is to be disbanded as a consequence of the museum's cost-cutting 1990–91 corporate plan. The visually exciting exhibition, which was a result of careful market research, has not met with universal praise, however. Its interactive video and spectacular effects have largely replaced traditional exhibits, something that a curator at another UK museum described as "appalling".

Henry Gee, London

SCIENTIFIC MISCONDUCT

Back to the drawing board

Washington

A two-year-old experiment in the investigation of scientific misconduct has blown up in the face of the US National Institutes of Health (NIH). The special office that NIH set up to investigate allegations of scientific misconduct has come under courtroom and congressional attack, and late last year a district judge in Wisconsin ruled that the office's procedures were illegal.

Now, as a host of similar lawsuits raise the prospect of NIH's misconduct rules being declared illegal in the rest of the country, the agency has decided to start the process again from the beginning. Officials say that within weeks NIH will publish in the *Federal Register* proposed new rules and procedures for the Office of Scientific Integrity (OSI), the department in question.

The move comes some three months after a crucial decision in the case of James Abbs, a researcher at the University of Wisconsin, who had been under investigation for alleged data fabrication (see *Nature* **349**, 95; 10 January 1991). The judge in that case ruled that NIH's investigation was illegal because OSI's internal procedures had been created *in camera*. The law demands that such procedures be published for public comment before they become law. At least two other lawsuits citing the Abbs decision have been filed since then (see *Nature* **349**, 357; 31 January 1991).

NIH intend to concede the issue and comply with the Administrative Procedures Act, says William Raub, acting director of NIH. The act requires that any proposed rules that could injure people outside government must be published with a comment period during which members of the public can suggest changes. Because OSI has taken the authority to go beyond simple investigations to the point of imposing sanctions (such as stopping a grant), it is subject to the act.

During the forthcoming rule-making process, which typically lasts for one to two years, OSI will have no legal procedures under which to operate. Nevertheless, NIH intend to continue investigating misconduct cases, but on a 'case-by-case basis' as they did before OSI was established in 1989.

"Before OSI was started, we thought we could handle investigations without written procedures", Raub says. "We thought the cases would be so few and so different that we wouldn't have to write things down. We were wrong."

OSI was created to bring order to the investigation process. But critics soon noted that the OSI, like NIH before it, tended to make up procedures as it went. (In the case of AIDS researcher Robert Gallo, for example, OSI asked the National Academy of Sciences to help it to set up a special advisory panel.) Faced with bitter complaints of arbitrary and capricious rule-making, OSI decided to formalize and publish its current rules (see *Nature* **346**, 9; 1990).

That, however, turned out to be a fatal mistake. A federal office with no rules may draw criticism, but an office that creates rules that conflict with the Administrative Procedures Act risks courtroom challenge, as NIH discovered.

Abandoning its existing procedures may lift the legal cloud over OSI for the moment, but it is unlikely to end its problems. If the office continues to impose sanctions, further lawsuits can be expected. Worse still, there is the possibility that researchers whose cases have already been closed may sue on the grounds that they were investigated under illegal rules. But Raub says that he can see no better way out. "Doing nothing is not an option", he says.

Robert Charrow, a Washington attorney, suggests a solution. If NIH would give up sanctions, he says, their legal problems could be over. "They ought to announce that all they will do is investigate, and then simply use the accepted hearing processes that are already in the books." NIH may eventually have to adopt that course. But until it is forced to abandon sanctions, the agency is eager to retain the right to halt research funding when the evidence indicates misconduct, if only for the sake of appearances.

Although the final decision on when or whether to publish new OSI procedures is now in the hands of the Justice Department, where lawyers are still considering their options, NIH officials are already writing the proposed rules. Raub says that the first draft will essentially codify the existing procedures. "We'll use that as a starting point", he says.

Concern in the scientific community is likely to centre on the issue of 'due process' — the protections and civil liberties guaranteed in US courts. Although NIH officials point out that OSI is not a court, several lawsuits have argued that an OSI investigation is as arduous as a criminal investigation, and can take far longer (in some cases more than two years). In particular, researchers have complained that the OSI rules do not give them the right to confront their accuser face-to-face and to see the evidence against them. But NIH, in an effort to protect whistle-blowers, intend to fight that point.

Once NIH publish the new OSI procedures, there will be a comment period (typically 90 days) during which the agency will accept any public input. OSI will then rewrite the rules in line with public consensus and legal concerns. Finally, a finished version of the rules will be published in the *Federal Register* and become law, ready for the next batch of misconduct cases. As for cases now under way or already closed, the courts appear to be the next battleground.

Christopher Anderson

CONFLICT OF INTEREST

Black eye for NIH

Washington

Officials at the National Institutes of Health (NIH) bungled a case of apparent conflict of interest, even after Congress pointed it out to them almost a year ago, a new investigation has revealed. The case itself is relatively pedestrian — Prem Sarin, deputy chief of AIDS pioneer Robert Gallo's NIH laboratory, is alleged to have illegally consulted with industry and set up a secret bank account to hide payments. But NIH's handling of the allegations raises serious questions about the agency's ability to police its own researchers.

At a hearing last week, Representative John Dingell (Democrat, Michigan), the firebrand chairman of the Energy and Commerce Committee's oversight and investigations subcommittee, asked NIH acting director William Raub, Gallo and two other NIH officials to explain their actions in the case.

Dingell noted that possible conflict of interest in Sarin's activities had first been brought to NIH's attention in April last year at a hearing on misuse of funds by Zaki Salahuddin, another researcher in Gallo's lab (see *Nature* **345**, 99; 1990). Salahuddin later pleaded guilty and was sentenced in district court (see *Nature* **348**, 8; 1990).

At the May hearing, Dingell revealed that a Food and Drug Administration (FDA) employee had written to the subcommittee to complain that Sarin had represented Responsif, a pharmaceutical company, at a 1985 FDA meeting, despite the fact that he was an NIH employee. Although Sarin, who was present at the Dingell hearing because he was Salahuddin's immediate supervisor, testified that his actions had been legal under NIH rules at the time, other NIH officials at the hearing were unable to confirm that and said that they would look into the matter.

What the officials actually did, an investigation by the congressional General Accounting Office (GAO) has revealed, was to ask Gallo to talk to Sarin about the matter. Sarin assured Gallo that "everything was taken care of", Gallo told the investigators. Dingell noted that this was exactly the way Salahuddin had successfully deflected suspicion when Gallo had questioned him on his own allegations of wrongdoing a year previously. "Apparently, these are magic words", Dingell wryly commented.

"It is discouraging", Dingell said, "that NIH did nothing to investigate the Sarin matter between the time of the April subcommittee hearing and December 1990", when his staff requested certain NIH documents that clearly showed irregularities in Sarin's consulting. Only then, when NIH were virtually handed evidence of wrongdoing, did officials investigate the case. NIH removed Sarin from his position earlier this year (see *Nature* **349**, 95; 1991) and the case is now being investigated by the agency's Inspector General and the US Attorney's office.

"It is not the business of the subcommittee to supervise the daily workings of the NIH. But if we have to do it, we will", Dingell threatened.

The most damaging evidence in the Sarin case revolves around an NIH fund — known as the Foundation for the Advanced Education in the Sciences (FAES) — that was used to support visiting scientists in Gallo's laboratory. According to the GAO investigators, Sarin set up a private bank account at a local bank with a similar name — FAES/ NERIC. These initials, however, stood for the "Family Account for the Education of the Sarin Children/Neil and Eric". Sarin instructed ASTA Pharma, a German pharmaceutical company, to make out a consulting cheque for $25,000 to FAES (an illegal payment, GAO said), which he deposited in the private account, along with other consulting payments. Sarin's lawyer, W. Neil Eggleston, says that Sarin has violated no laws, but he declines to explain the details of the questioned bank account.

NIH did not spot the violations, Raub testified, because officials had not thoroughly examined Sarin's files and were not sure that he had been given correct advice when he sought approval for the consulting. "In hindsight, the level of trust was too high", he said. "One of the things we need to develop across NIH is a greater talent for selective suspicion."

Gallo testified that, although he had been asked to question Sarin about the allegations, he could do little more than accept Sarin's denial.

"If the subcommittee and the NIH know about the allegations of misconduct, I don't know what I'm doing investigating it", Gallo argued. "I'm a scientist, not a policeman."

It is not clear exactly what NIH have done to prevent and investigate conflict of interest abuses in the future. "Do you still go by the same seat-of-the-pants review process that brought about this sorry situation?", Dingell asked Richard Adamson, Gallo's superior at the National Cancer Institute (NCI).

"No", Adamson responded. He was, however, unable to specify any significant new protection in the NIH procedures prompted by the Sarin case. Raub testified that, since the Salahuddin hearing, NIH have improved its review of applications for outside consulting and started an NIH-wide inventory of equipment. But most effort seems to have been spent in ensuring that existing procedures are being followed. NCI employees have been given seminars on existing conflict-of-interest rules, and outside companies will be sent copies of the relevant regulations, so that they can watch for violations as well, Raub said.

Christopher Anderson

EUROPEAN RESEARCH

Commission bids for power

London

As the European Communities (EC) member states debate plans for greater economic and political unity, the European Commission has launched a bold plan to wrest control over the EC's research programme from the Council of Ministers — the periodic meetings of member states' research ministers.

The move would decrease the influence that individual member states have on the precise direction of EC research. This alarms many of the member states' permanent representatives in Brussels, who are embroiled in a dispute with the Commission's controversial vice-president in charge of research, Filippo Pandolfi, over the EC's future support for basic science.

The Commission, the civil service of the EC, is responsible for drawing up the EC's Framework research programmes, in consultation with the member states. But the go-ahead for individual component programmes within each Framework currently depends on the research ministers giving their approval.

The Commission's bid for increased power is put forward as a proposal to the Intergovernmental Conference that is considering plans for greater European unity. The Commission argues that each Framework should be approved in a single step, rather than individual component programmes being subject to scrutiny by the Council of Ministers after the overall budget is agreed. Under the plan, the Commission would then be able to implement the individual programme itself. This would give the Commission a large degree of control over a huge sum of money: a budget of 5,700 million ECU (about £4,000 million) was set last year for the EC's third Framework programme.

The proposal was considered by a meeting of member states' permanent representatives in Brussels late last month. Most are said to have been unenthusiastic. But the Commission has one, superficially appealing, argument to support its plan to streamline the decision-making process: the third Framework programme has become hopelessly bogged down in discussions between the Commission, the European Parliament and the member states. After almost a year, no individual programmes have yet received the research ministers' final approval.

But Pandolfi's critics say that the Commission is to blame for the delay. The problem, they argue, stems from Pandolfi's decision to present the outlines of 13 component programmes within the third Framework at one go. Previously, the European Parliament, the member states' research ministers and their permanent representatives in Brussels were able to consider programmes in smaller, more manageable batches.

To compound the administrative nightmare, the Commission was last year forced to rewrite the 13 programme outlines, because they contained too little detail. Even now, the "human capital and mobility" programme, which will take over EC support for basic scientific research, is the subject of a bitter row between Pandolfi and the member states' representatives. Pandolfi wishes to spend most of the 518 million ECU (about £350 million) on a large postdoctoral fellowship scheme, which his opponents argue vastly exceeds the demand (see *Nature* **349**, 556; 14 February 1991).

In the light of the current chaos, Sir Peter Swinnerton-Dyer, chairman of CODEST, the committee that oversees EC support for basic research, says he cannot see the member states' research ministers agreeing to the Commission's proposal. The European Parliament is also likely to fight the plan. The parliament can influence EC research programmes through its decisions on the EC's annual budget, and is wary of any extension of the Commission's role. With this opposition, the Commission's plan is unlikely to be accepted in its present form. Nevertheless, with the EC's constitution effectively 'up for grabs' as the member states debate greater European unity, it is possible that the Commission may win some concessions and consolidate its role in directing EC research.

Peter Aldhous

UK UNIVERSITIES

New CVCP chairman

London

UK university vice-chancellors have chosen David Harrison from the University of Exeter to head the Committee of Vice-Chancellors and Principals (CVCP) from 1 August. Harrison, a chemist, is the second vice-chancellor to be appointed to the post in recent months. The first, Graeme Davies from the University of Liverpool, withdrew when he accepted the position of chief executive of the Universities Funding Council (UFC) (see *Nature* 349, 443; 7 February 1991). The two men face a difficult task improving strained relations between their respective bodies.

Harrison — tough job ahead.

Peter Aldhous

INTERNATIONAL COMPETITION

MITI moves to open patents

Tokyo

Foreign companies that join research and development projects funded by Japan's Ministry of International Trade and Industry (MITI) should soon be able to hold patent rights arising out of the research. MITI hopes that the move will encourage foreign companies to take part in research and development projects in Japan.

Until now, all patent rights arising from projects funded directly by MITI have been held by the government, and participating companies — both foreign and Japanese — have to pay licence fees to use technology arising from their own research. In the case of projects funded by the New Energy and Industrial Technology Development Organization (NEDO), a semi-government organization affiliated to MITI, companies can hold up to 50 per cent of patent rights, but they still have to pay licence fees.

Under a proposed new law, which MITI submitted last week to the cabinet, companies will have access to patents from NEDO and MITI projects free of charge or for a "small fee". The powerful ministry has previously spurred Japan's economic success through aggressive national projects, and the patent legislation is seen as the first step in a new MITI policy of promoting international research on new technology among developed nations.

The new "technoglobalism" policy, which MITI announced at a meeting last year of the Organisation for Economic Cooperation and Development, is designed to counter a perceived "technonationalism" in the United States, whereby the United States tries to protect its own technological advances from countries such as Japan that are making rapid progress in developing high technology.

The move to introduce the new legislation was in part spurred by a large-scale project launched by MITI through NEDO in 1989 to develop a "super/hypersonic transport system" to follow the Anglo-French Concorde. US and European companies, including Rolls-Royce of the United Kingdom, United Technologies, Pratt and Whitney, and General Electric of the United States, and SNECMA of France, wanted to join the project, in which MITI will invest ¥28,000 million ($200 million) over eight years, but they were discouraged by the regulations governing patent rights. Agreement for these companies to join was finally reached a few weeks ago, after months of wrangling over patent rights.

The legislation will bring Japan more closely into line with France and Germany (see table). In the United States, foreign companies cannot hold patent rights arising out of government-funded research, but they can license technology free of charge if they participate in a project. In the United Kingdom, the government retains patent rights for inventions that arise from contract research, but companies can hold patents when the government only provides subsidies.

MITI official say they are optimistic that the Science and Technology Agency will soon follow suit with more general legislation covering all Japanese national research projects.

David Swinbanks

PATENT RIGHTS FOR COMPANIES PARTICIPATING IN
GOVERNMENT-FUNDED RESEARCH AND DEVELOPMENT PROJECTS

Country	Holder of rights	Licence fee
Japan		
National project	Now: government Revised: 50 per cent or more government; up to 50 per cent for companies	Fee required Free or small fee
NEDO project	Now: 50 per cent or more NEDO; up to 50 per cent for companies Revised: as above	Fee required Free or small fee
US		
US companies	Companies	Free
Non-US companies	Government	Free
UK	Government	Free
France	Companies	Free
Germany	Companies	Free

INTERNATIONAL COLLABORATION

Euro-laser plans on ice

London

European science administrators have shelved plans to build a laser facility that would compete with the best the United States and Japan have to offer. The proposed ten-year collaboration among Britain, Germany, France, Italy and Spain was aimed at producing a 100-kilojoule ultraviolet laser that would give European physicists a state-of-the-art machine with which to study plasma behaviour and the fusion of hydrogen isotopes (see *Nature* 346, 503; 1990). But Britain, France and Germany have now decided that the project is too expensive.

The British contribution is among the projects axed to ease the financial difficulties at the Science and Engineering Research Council (SERC, see *Nature* 349, 551; 14 February 1991), and the high costs of reunification are currently restricting German spending on international science projects. The original proposal would have cost up to £25 million a year and would have required first building two smaller machines to test rival laser technologies before constructing the final 100-kJ facility.

Michael Key, from SERC's Rutherford Appleton Laboratory and one of the authors of the European laser proposal, says the project may be revived when the economic situation across Europe improves. Key takes heart from the example of the European Synchrotron Radiation Facility, now being built in Grenoble, which was approved only after several years of discussion. In the short term, Key says the priority now is to upgrade existing lasers around Europe, and to encourage their shared use, perhaps through funding from the European Communities.

Peter Aldhous

PREVENTIVE MEDICINE

AIDS control through cultural heritage

New Delhi

Ancient scriptures and religious books will be searched for messages that can be used to control the spread of AIDS in a new programme launched by the Indian Council of Medical Research (ICMR). The council says that Western-style educational campaigns, which often rely on television and printed booklets, will have little effect on Indians, whose life style is shaped more by religion than by science.

A. S. Paintal, director general of ICMR, notes that the United States and African countries have had difficulty controlling the spread of AIDS despite printed information and television advertisements. India has an advantage over Western countries in that it has a rich cultural heritage and scores of scriptures that already contain the necessary prescriptions for an AIDS-free society, Paintal says.

This led to the idea for the campaign, whose theme is "Protect yourself from AIDS through cultural heritage". The hope is that AIDS messages based on religion will be more easily accepted by an average Indian than the same messages given as bland scientific facts through health bulletins. Paintal says that Indian scriptures are replete with messages preaching moderation in sex and describing prostitution, homosexuality and adultery as sin. "All we are saying is, make our people aware of these teachings", Paintal says. "Just telling them about safe sex will not help in changing their attitudes."

K.S. Jayaraman

GEOSCIENCES

Predicting earthquake risks

San Francisco

For the first time, scientists are planning to assemble a comprehensive model of the earthquake hazards for a single earthquake-prone region. Focusing on Southern California and integrating 30 years' worth of data from disparate sources, researchers at the newly established Southern California Earthquake Center (SCEC) hope to improve earthquake forecasting and help to predict the strength of ground motions in specific locations in the Los Angeles area.

In particular, this 'master model' should help emergency planners and urban developers, as well as Earth scientists, to attain a better understanding of exactly what earthquake hazards exist in which areas of Southern California.

Although scientists at scores of institutions study California's frequent tremors, no one has ever attempted comprehensive seismic predictions or maps of hazard zones for the region, says SCEC's executive director, Thomas Henyey. But with $5 million from the National Science Foundation (NSF) and the United States Geological Survey (USGS) in its first-year budget, and an expected 5–11 years of support as an NSF Science and Technology Center, the centre plans to change that. Located at the University of Southern California, it will draw on scientists from seven universities and the USGS.

The model will undoubtedly require much new research, but adequate data already exist to develop rudimentary hazard analysis maps, Henyey says. And with refinement, the maps may serve very practical purposes. "Soon, we hope you'll be able to come to us as a developer and ask what is the probability of a quake on a particular property and how strong the ground motion will be", he says. "Our goal is to give you our best description of the quake hazard that will be location-specific."

The database will also be used by the National Earthquake Prediction Evaluation Council to improve both short-term and long-term earthquake predictions, says California state geologist James Davis, a member of the council. In addition, SCEC will probably collaborate with the National Center for Earthquake Engineering Research at the State University of New York in Buffalo, another NSF-funded centre.

Henyey expects the centre to take at least two years to integrate existing earthquake-related data into a useful form. The research areas that will be important to the centre's master model include the history of fault movements, earthquake statistics, patterns of strain buildup and crust deformation, physical properties of earthquake sources and descriptions of surface shaking in response to seismic waves. Once the database is complete, it will be used to help guide the centre's future research directions, but SCEC is already reviewing the nearly 40 research proposals it has received from interested scientists, and it expects to make funding decisions on them in the next few weeks.

The SCEC may have a Northern California counterpart soon, Davis says. Researchers in the San Francisco Bay area are considering development of a similar organization for their region, which, like Southern California, is populous, prone to earthquakes and well studied by Earth scientists.

SCEC is set up as a 'center without walls' — an organization of people in many institutions rather than in a single location. In addition to USC and the USGS, the centre's participants include the California Institute of Technology, the University of California at the campuses of Los Angeles, San Diego, Santa Barbara and Santa Cruz, and Columbia University's Lamont-Doherty Geological Observatory in New York.

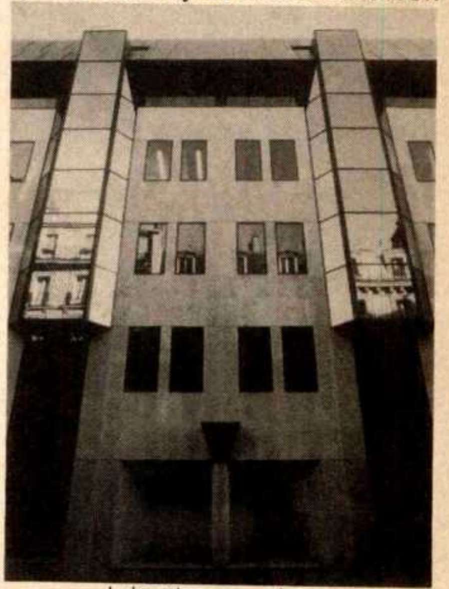
Elizabeth Schaefer

PASTEUR INSTITUTE

New building defies the metro

Paris

The French Prime Minister, Michel Rocard, last week inaugurated a new retrovirus building at the Pasteur Institute in Paris. Costing FF100 million, the 4,685-square-metre building was paid for entirely by funds raised by the institute since 1987.



Laboratory on springs.

There are ten laboratories with space for 120 researchers and the same number of technicians and administrators, mostly working on AIDS.

The building incorporates some unusual features. The whole structure, which weighs 12,800 tonnes, is supported on 246 massive springs whose function is to absorb vibrations from a Paris metro line that runs just a few metres from the foundations. And to make sure viral material does not escape into the atmosphere, each of the four maximum-security laboratories has total air filtering, special extractors and autoclaves, and is kept below ordinary atmospheric pressure.

Since 1984, spending on AIDS research at the Pasteur has increased by a factor of six and accounts for about one-fifth of the FF600-million annual budget in 1990.

Peter Coles

INTERNATIONAL COLLABORATION

Reforms sought from UK Treasury

P 36,666

London

The Treasury should protect the UK research councils when the cost of participating in international research programmes escalates owing to fluctuating currency exchange rates, according to the House of Lords Science and Technology (S&T) Committee.

When an international collaboration is based overseas, the British contribution must be paid in the host country's currency. The S&T committee's report on international collaboration in science, published yesterday (13 March), says that the Treasury, rather than the research councils, should pay for cost increases caused by the pound's value dropping by more than two-and-a-half per cent against a pre-determined value. The Lords describe the current situation, in which the research councils must play the international currency markets in an attempt to minimize the impact of exchange rate fluctuation, as "grotesque".

If accepted by the government, the proposal would ease a long-standing concern of the Science and Engineering Research Council (SERC), which has almost one-third of its annual budget tied up in international projects. The committee notes that SERC was forced to bear the cost of a one-third real-terms rise in the UK subscription to CERN, the European particle physics laboratory in Geneva, between 1985 and 1988.

The S&T committee also attacks the Treasury's practice of reducing the domestic British spending on science to compensate for money that UK scientists win from the European Communities' research programmes, joining a chorus of criticism from other all-party parliamentary committees (see *Nature* **346**, 305; 1990 & **349**, 183; 1991). This 'attribution' means that, often, only one-third of Britain's spending on EC research represents a real addition to the domestic spending on science. The S&T committee's report notes that neither France nor Germany operates a similar system.

Peter Aldhous

Complexity still running

SIR — Your correspondents (*Nature* **348**, 280; 1990) trying to tackle my question about runaway complexity (*Nature* **347**, 704; 1990) have missed the point, which concerns the inverse relationship between morphological complexity and population size. Invoking the eukaryote/prokaryote distinction is not 'the simple answer' as offered by Philip Wood, because the origin of eukaryotes from prokaryotes is itself only part of the more general problem. I accept, of course, the claim of great biochemical versatility, adaptability and general success of today's prokaryotes, made on their behalf by an unusually articulate variant of *E. coli* (*Nature* **349**, 97; 1991).

One might loosely define more complex organisms as having more morphological features whose presence is ascribed to the sometime action of directional selection. But more complex organisms generally have smaller population sizes and tend to have longer generation times, so *en route* to increasing complexity there should be fewer and fewer opportunities to build up any new complex feature step-by-step in a sustained series of selection events. Most scenarios to generate the phenomenal complexity of even the simplest metazoans seem to require vast numbers of successive selection events acting on particular traits; that is, episodes of persistent directional selection for one feature after another. The awesome morphological complexity of organisms such as vertebrates that have far fewer individuals on which selection can act therefore remains somewhat puzzling (for me at least), despite the geological timescales available and notwithstanding the insights provided by John Bonner in his book *The Evolution of Complexity* and Richard Dawkins' demonstration of the power of selection in *The Blind Watchmaker*. Could there therefore be a tendency, as yet neither fully recognized nor understood, for more complex organisms to throw up ever wider ranges of viable variation, including occasional novel but well-integrated features that give every impression of sustained selection? If so, any such tendency for 'runaway complexity' may have had a relatively free rein of expression during the metazoan radiations in the late Precambrian and early Cambrian.

In general, we know much about frequency-changing processes in evolution but still very little about how new traits and forms arise, as recently admitted by Endler and McLellan in a comprehensive review (*A. Rev. Ecol. Syst.* **19**, 395–421; 1988).

PETER R. SHELDON

Department of Geology,
University of Wales,
PO Box 914,
Cardiff CF1 3YE, UK

Origins of AIDS

SIR — Why do Western analysts need always to invoke exotic sexual practices by Africans in order to explain transmission of HIV-related viruses from monkeys to man when far likelier routes for transmission are readily apparent? In an otherwise excellent letter on the origin and spread of AIDS, A. Karpas (*Nature* **348** 578; 1990) cites a paper by F. Noireau (*Lancet* **i**, 1498; 1987) suggesting that elaborate sexual rituals involving human genitalia and monkey blood are a likely mode of trans-species transmission. Yet in the same letter, Karpas discusses monkey-virus transmission to (Western) laboratory workers. Suggestions of deviant sexual behaviour are no longer evoked — simple monkey bites are now considered sufficient to explain transmission. Man has been hunting, killing and eating monkey in Africa for millennia. This has undoubtedly led to countless bites, cuts and abrasions that have provided ideal opportunities for blood-borne inoculation. Scientific writers should leave descriptions of the more exotic sexual rituals to either anthropologists or the glossier pages of other types of publication, rather than *Nature*.

JOHNJOE MCFADDEN

Department of Microbiology,
University of Surrey,
Guildford, Surrey GU2 5XH, UK

Genome project

SIR — Biologists should be disquieted by the special pleading of Walter Gilbert for the genome project (*Nature* **349**, 99; 1991). The assumptions he makes are odd. For instance, he seems to imply that, once we have identified the genes in an organism, we have obtained virtually all the basic information required to explain biological phenomena. The limiting factor is understanding not only the gene structure but also the gene expression, which varies in a most complex way. Monod made great advances towards the understanding of gene expression but only to one case, namely expression of the β-galactosidase gene.

But consider the problem when one becomes seriously ill with, say, a virus infection. Although the genes are the same, a cascade or even catastrophic series of changes occurs in the body functions, or gene expressions, of which man is essentially ignorant. The 'new paradigm' should be addressed not merely to gene structure but to elucidation of the integrated functions of genes, enzymes, membranes and metabolites in the whole organism. Only such an extended paradigm can lead to explanations of the biological phenomena that Gilbert lists in his preamble. Incidentally, formulating a hypothesis and testing it experimentally is not due to start only when we have identified the genes. A review of the literature will show that biologists have always effectively used that principle.

The use of 'kits' and computers on the grand scale for the human genome project is an admirable ambition. But let us recognize its limitations and ensure that it does not distort the rest of bioscientific research. The attack on the gene expression problem is much more challenging and difficult. The resort to the kit and the computer routines seems to be an example of that well-known human failing — taking the line of least resistance.

S. JOHN PIRT
(Emeritus professor,
King's College London)
50 Chartfield Avenue,
London SW15 6HG, UK

Grants as status

SIR — Ray Iles (*Nature* **349**, 188; 1991) is lucky to be able to court the biotechnology companies. Obvious sources of funding in anaesthesia are the pharmaceutical companies — except that their money does not count in the scheme of 'brownie points' erected by those who judge our worth, to whom the acquisition of grant money seems the most important criterion. However, in these days of shrinking resources one would imagine that doing research for the least cost would be a laudable aim; yet one scores more highly by securing grant money, even if the project can be done without its need. It is an intellectually bankrupt notion to suppose that research that is more expensive is necessarily better. I pity the theoreticians; or perhaps they secure grants for supplies of expensive fountain pens and pure vellum on which to write.

NEVILLE W. GOODMAN

University Department of Anaesthesia,
Southmead Hospital,
Bristol, BS10 5NB, UK

Ordered layers

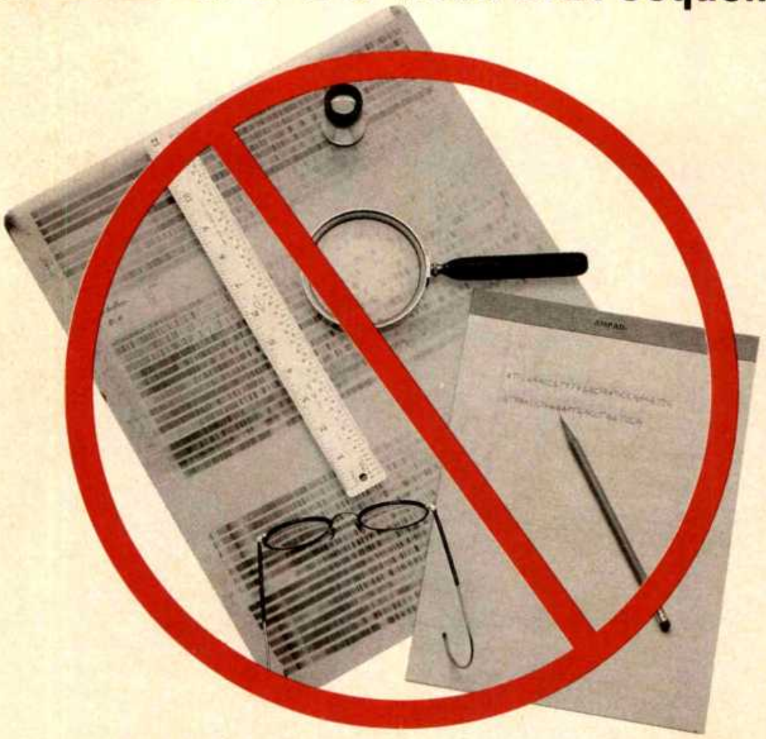
SIR — The intriguing piece by Daedalus on ambitious chemists and micromagnetics (*Nature* **348**, 588; 1990) came in the middle of a related discussion here. I have supposed that, if the vapour phase deposition of organic compounds (such as porphyrins, phthalocyzanines and other aromatics) which are able to sustain a sizeable induced ring current were to be carried out in a high magnetic field, then the induced magnetic field would influence the mode of deposition. With care it might be possible to get nicely ordered layers in this way; and clearly the experiment could be done with the external field at various orientations to the direction of gaseous molecular flow.

Has this sort of experiment been done? If not, would anyone be prepared to do it? If not in the United Kingdom, then maybe in Japan? I would be happy to supply suitable porphyrins and phthalocyanines.

R. BONNETT

Department of Chemistry,
Queen Mary and Westfield College,
Mile End Road, London E1 4NS, UK

CAUTION: This May Change The Way You Read DNA Sequencing Films



The Discovery Series™ Model DNA 35 Automatically Reads DNA Sequencing Films

Hardware, Software and how it will change the way you do DNA Sequencing Reading.

To compete in today's world, you can use the old tools...or...choose today's technology. The Discovery Series™ model DNA 35.

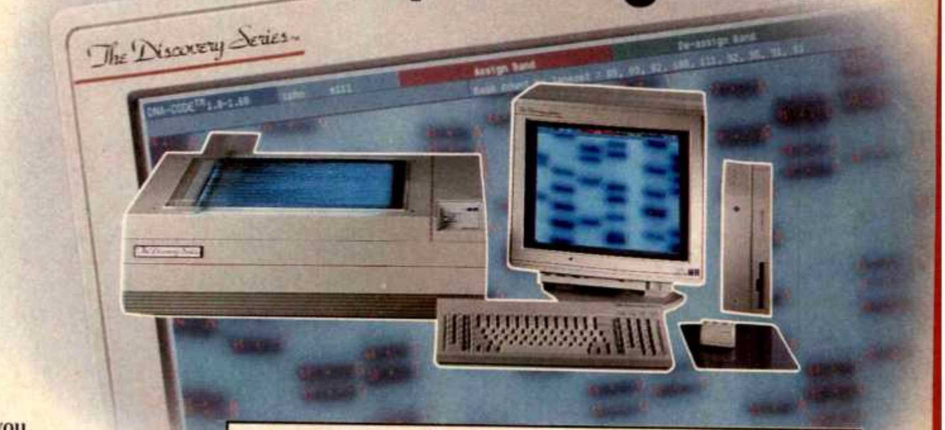
- It gives you the power to scan, read, edit and compare 3,000 bases in 30 minutes.
- It fits on a desktop or lab bench.
- The new DeskTop™ scanner provides the image to work with.
- DNA Code® v2.0. A new software release with more sequence reading performance to turn *your* image into data.
- New 16 MIPS UNIX® minicomputers work as fast as you want.
- It also comes with Quantity One® – 1-D gel and dot/slot blot software.

Scan your film in seconds at 63.5 micron resolution and watch the digitized image flow onto your computer screen in real-time. The combination of our new DNA sequence reading software, a new high-resolution scanner, and UNIX® based computers gives you real-life, magnified graphics to work with. Automatic base-calling and interactive editing tools finish the task.

1-D gels, dot & slot blots? Yes, the model DNA 35 can quantitate all types of 1-D gels and films.

You're doing more than DNA Sequencing. That's why the DNA 35 also comes with **Quantity One**,® the software package for the quantification of all types of 1-D applications and dot/slot blots.

UNIX® is a trademark of AT&T Laboratories. Quantity One® and DNA Code® are trademarks of PDI, Inc.



Application	Software Package
DNA sequence reading	DNA Code®
1-D gel quantification	Quantity One®
Dot and slot blot analysis	Quantity One®

Call PDI toll-free today for more information.

Call **1-800-777-6834** (USA only) to set up a demonstration of The Discovery Series™ model DNA 35 sequencing DNA in *your* lab. We look forward to working with you to turn *your* data into results.

pdi

formerly protein databases inc

protein + dna imageWare systems℠

405 Oakwood Road, Huntington Station, New York 11746
Phone: 516-673-3939, 1-800-777-6834, Fax: 516-673-4502

Reader Service No.594

In the Benelux countries: DALTON, bv., PO Box 154, NL 5140 AD Waalwijk, The Netherlands, Tel: (0)4160-31225, Fax: (0)4160-41265
In Japan: Toyobo Co. Ltd., Osaka, Japan, Tel: 06-348-3786, Fax: 06-348-3322
In Germany, Austria, Switzerland and Eastern Europe: Sud-Laborbedarf, GMBH, D 8035, Gauting, FRG, Tel: 089 850 6527, Fax: 089 850 7646

ATTRACTS

READERS,

RESPONSES,

SALES

thermocycler heating units freezers incubators electro-
phoresis test tubes primers glycoproteins spectrometers
growth factors antibodies test kits software nucleic acids tissue
grinders image analysis equipment pipettes lipids centrifuges amino acids
water purifiers robotic liquid handling
tissue culture labware ovens
western blotting ECL
centrifuges biochemicals
DNA purification kits
antibodies antiserum
designer genes microplate
reader HPCE DNA sequencers
lab furniture peristaltic pumps
biocatalysts hormones microscopes
data recorders agar cell cultures cell counters
Taq polymerase pH meters glove boxes bottles spectro-
photometers hardware cytokines radiographs enzymes probes
chromatography tissue culture growth factors gels peptides ovens
data recorders agar cell cultures cell counters
reagents bovine serum HPLC centrifuges biochemicals
DNA synthesiser shakers stirrers
NATURE UK PRODUCT REVIEW
lab technicians
research scientists
product specialists
data analysts
R & D managers
technical directors
job hunters
chief technicians
lab directors

USE THE FORCE – ADVERTISE TODAY!

To book space call Julie Bryant 071 872 0103 or
fax 071 240 2408

Send your press releases to: Nuala Moran,
UK Product Review, Nature, Macmillan Magazines Ltd,
4 Little Essex Street, London, WC2R 3LF

Reader Service No.31

CIRCULATION NOW 12,00

Presenting the latest techniques and applications for the most promising therapeutic monoclonal antibodies

Coming Soon

Therapeutic Monoclonal Antibodies

Edited by C.A.K. Borrebaeck, D.Sc., *University of Lund, Lund, Sweden* and J.W. Larrick, M.D., Ph.D., *Genelabs, Inc., Redwood City, CA*

As commercial production of therapeutic monoclonal antibodies becomes a reality, researchers and scientists require accurate, up-to-date information to keep abreast of this rapidly moving field. There are few reliable sources of information available, particularly in one convenient publication. **Therapeutic Monoclonal Antibodies** fulfills the need for a single reference covering the latest advances in both preclinical and clinical work on monoclonal antibodies. Written by an international team of leading experts, this exhaustive work details the latest progress made in the clinical application of monoclonal antibody technology.

CONTENTS & CONTRIBUTORS:

The Use of *In Vitro* Immunization, Cloning of Variable Regions and SCID Mice for the Production of Human Monoclonal Antibodies; C.A.K. Borrebaeck, L. Danielsson, M. Ohlin, J. Carlsson, R. Carlsson • Construction of Recombinant Therapeutic Monoclonal Antibodies; J.W. Larrick, J.V. Gavilondo, M.J. Coloma, K.E. Fry • Antibody-Mediated Cancer Diagnosis and Therapy; J. Bator, C.L. Reading • Current Status and Future Perspectives of Chemoimmunoconjugates for the Treatment of Cancer; R.A. Reisfeld, M. Schrappe • Gangliosides as Targets for Monoclonal Antibody Therapy of Cancer; R.F. Irie, M.H. Ravindranath • Mouse Antibodies to Carcinomas; K.E. Hellstrom, I. Hellstrom • Therapeutic Implication of Monoclonal Antibodies against Multidrug-Resistant Tumor Cells; T. Tsuruo, H. Hamada • Radioimmunotherapy with 90Yttrium-Anti-Idiotype Monoclonal Antibody; B.A. Parker, S.E. Halpern, I. Royston • Immunoconjugates of a Protein Synthesis-Inhibiting Drug; A.C. Morgan, Jr., F.T. Comezoglu, R. Manger, B.B. Jarvis, P.G. Abrams, G. Sivam • Human Monoclonal Anti-Idiotypic Antibodies against a Murine Monoclonal Antibody (Mab17-1A) as an Anti-Tumor Vaccine; M. Steinitz, S. Tamir, J.-E. Frodin, H. Mellstedt • Uses of Human Monoclonal Antibodies to Human Cytomegalovirus and Varicella-Zoster Virus; S.K.H. Foung, P.A. Bradshaw, D. Emanuel • Development of a Human Monoclonal Antibody against Cytomegalovirus with the Aim of Passive Immunotherapy; Y. Masuho, Y.-I. Matsumoto, T. Sugano, T. Tomiyama, S. Sasaki, T. Koyama • Characterization of Human Anti-Cytomegalovirus Monoclonal Antibodies; P.H. Ehrlich, L. Ostberg • Immunoprotective Capacities of Human and Murine Monoclonal Antibodies Recognizing Serotype Specific and Common Determinants of Gram-Negative Bacteria; A.B. Lang, U. Bruderer, E. Fuerer, J.W. Larrick, S.J. Cryz, Jr. • Pharmacokinetics of Xomen TM-E5 in Animals and Humans; A.H.C. Kung, E.A. Saria, N. Wedel, P.W. Trown • Randomized Placebo-Controlled Study of E5 Monoclonal Antiendotoxin Antibody; K. Gorelick, P. Scannon, J. Hannigan, N. Wedel, S. Ackerman • Human Monoclonal Antibodies against Human Red Blood Cells; P. Rouger, D. Goossens • CD7 Monoclonal Antibodies; P. Amlot, S. Cammisuli • Large Scale Production of Monoclonal Antibodies; C. Glad, I. Nilsson • European Regulatory Issues; J. Brown

November 1990, approx. 320 pp., hardcover 0-33353544-8 £49.00

Send orders to Globe Book Service Ltd., FREEPOST, Stockton House, 1 Melbourne Place, London WC2B 4LF, UK. Or call: 01-379-4687.

We accept Access/MasterCard, Visa, Diners' Club and American Express. Please add £2.00 postage and packing. FREEPOST applies to UK only. (Globe Book Services is a division of Macmillan, Publishers, Ltd.)

BIOLOGICALS AVAILABLE FROM THE NATIONAL CANCER INSTITUTE (NCI)

The repository of the Biological Response Modifiers Program (BRMP), Division of Cancer Treatment (DCT), NCI, NIH, announces the availability of recombinant human lymphokines IL-1α, IL-1ß and IL-2 and the monoclonal antibodies 11B.11, against mouse IL-4 and 3ƵD, against human IL-1ß.

Use of these materials is limited solely to *in vivo* and *in vitro* basic research studies and is not intended for administration to humans.

The lymphokine materials are aliquoted in 100 μg amounts ($>10^6$ units) and are available to investigators with peer-reviewed support. However, manufacturers' restrictions prohibit distribution of these materials to for-profit institutions or commercial establishments.

The monoclonal antibodies are available to peer-reviewed investigators, for-profit institutions or commercial establishments. The 11B.11 antibody is available in either 3 or 50 mg vials. The 3ƵD antibody is available in 5 or 20 mg amounts.

Investigators wishing to obtain any of these materials should send requests to:

Dr. Craig W. Reynolds
Biological Response
Modifiers Program
NCI-FCRDC
Building 1052, Room 253
Frederick, MD 21702-1013

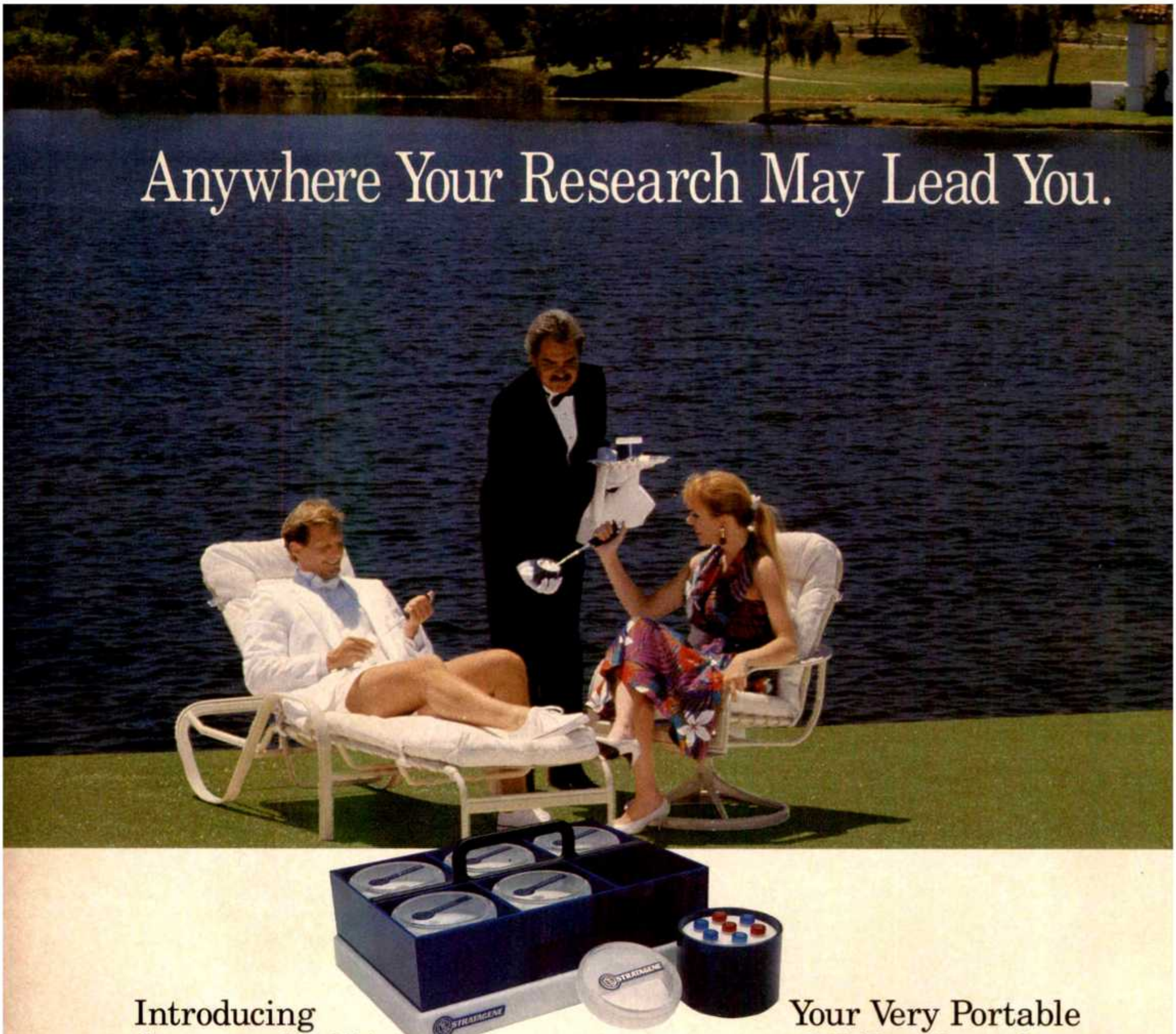
All requests should be accompanied by:

(1) A brief paragraph outlining the purpose for which materials are to be used, (2) the amount desired, (3) description of investigator's peer-reviewed support. Recipients will be required to sign a Materials Transfer Agreement and to pay shipping and handling costs. Please allow 4 to 6 weeks for delivery.

National Cancer Institute —
Frederick Cancer Research Facility

Reader Service No 41

Anywhere Your Research May Lead You.



Introducing Stratacooler® Lite

Your Very Portable -20° Benchtop freezer

Reach for the Stratacooler® Lite freezer. Or better yet, go for a six pack of Lites. Wherever your research may lead you, the Stratacooler Lite is designed to maintain subzero temperatures. Within your -20°C freezer the Stratacooler Lite insulates your enzymes from periodic temperature increases due to open freezer doors, frost-free cycles, or power loss. Your Stratacooler Lite will maintain subzero temperatures for up to two hours.

The Stratacooler Lite's portable design is ideal for the laboratory or for research on the go. The Stratacooler Lite is easily carried in one hand and is perfect for safe transportation of your valuable enzymes.

The Stratacooler Lite accommodates 7 standard microcentrifuge style tubes and is thoroughly tested and guaranteed to perform to specifications. Stratagene's LiteRack which holds 6 Stratacooler Lites is also available.

Please call for distributor information and details on Stratagene's full line of innovative products.

Product	Cat. #
Stratacooler Lite	400012
LiteRack	400017

Reader Service No.526

The Stratacooler® II that accommodates 32 standard microcentrifuge style tubes is also available. Cat. # 400002.

STRATAGENE®

Corporate Headquarters:
11099 North Torrey Pines Road
La Jolla, CA 92037
Ordering: 800-424-5444
Tech. Services: 800-548-1113
FAX: 619-535-5430
TELEX: 9103809841

Stratagene GmbH
Postfach 105466
D-6900 Heidelberg
(Federal Republic of Germany)
Telephone: (06221) 40 06 34
Telefax: (06221) 40 06 39

Stratagene Ltd.
Cambridge Innovation Centre
Cambridge Science Park
Milton Road
Cambridge CB4 4GF
Telephone: (0223) 420955
Telefax: (0223) 420234
Telex: 81417 INNCEN G

Classical liquid in a box

A simulation of a fluid of interacting particles in a box much narrower in one dimension than the other two reveals a curious bimodal density distribution along the short dimension — and provides a telling example of Poisson statistics.

THAT the perennial question of whether fashions for problems of a particular kind precede or follow the arrival of means for their solution will never be settled is nicely illustrated by the recent interest in smallish clusters of atoms or molecules. The recognition of small clusters in atomic or molecular beams almost coincided with the point at which the techniques of molecular physics could be turned in that direction with some chance of success.

That, no doubt, is one reason why the problems of molecular systems whose dimensions are essentially atomic are now all the rage. But that does not diminish their high interest, which is that of throwing light on the transition between the properties of a collection of isolated molecules and a bulk aggregate thereof. But now there is a model calculation of a system of this kind, essentially a bulk liquid consisting of classical atoms, in which only one of the three physical dimensions is of an atomic scale. Its outcome is full of surprise.

The problem, definable theoretically if unattainable in the machine-shop, is that of a liquid enclosed between infinite parallel plates which are separated from each other by only three atomic diameters. It is the problem of a fluid in a flattened capillary tube. There are obvious connections with that of a thin layer of fluid adsorbed on the surface of a solid as a two-dimensional gas (but the constraints are greater), as well as with the behaviour of a fluid in a porous structure (but "micropore" would be a better word).

J. G. Powles, A. Dornford-Smith and W. A. B. Evans from the University of Kent at Canterbury (England), who have a reputation in the solution of static versions of problems such as this, now say that their interest is in the dynamics of atomic motion in such a system (*Phys. Rev. Lett.* **66**, 1177–1180; 4 March 1991).

Describing the problem is easier than solving it. Pairs of atoms are supposed to interact by means of a Lennard–Jones potential — the standard combination of an attractive force varying with the sixth power of the inverse separation and a repulsive force varying similarly with the twelfth power (which dominates at short distances). The 'walls', simulated as an external potential applicable to all atoms between them, repel all atoms according to the ninth power of their distance, thus preventing what might be called condensation on the surface.

The bare bones of static equilibrium are easily guessed at. If the fluid were a perfect gas, so that the mutual interaction of pairs of molecules vanishes, but if the repulsion of the walls persists, there will be a smooth distribution of molecules within the slab of space between the walls, with maximum density midway between them. Increasing temperature (or giving the atoms kinetic energy) makes the distribution flatter. But what happens if the mutual interaction is switched on? Then there is a relative concentration of molecules in two layers on either side of the midpoint (which is only, in this model, 1.5 atomic diameters from each wall).

Handwaving will get one almost there. Atoms are repelled both by the walls and by those of their own number lying closer than just over one atomic diameter. (The energy minimum of the Lennard–Jones potential is at 1.12 times the number taken to be the diameter.) So a density distribution in which atoms were crowded near the midpoint would be unstable relative to a bimodal distribution, but less so at increased temperature.

In practice, these properties are arrived at by numerical calculation. Powles, Dornford-Smith and Evans put 1,020 atoms in a simulated box and follow what happens to them. As it happens, the two long dimensions of the box turn out to be just over 23 atomic diameters — far from infinite, but probably sufficient for the purposes. It turns out that there is little of interest to say about the motion of atoms parallel with the confining walls; even if one of them should embark from a place where the density is a maximum, only rarely will it thread its way through the relatively dense gas at that exact distance from one wall. So classical diffusion is a sufficient picture of what happens.

The fun comes in the direction perpendicular to the wall. What emerges from individual trajectories is that atoms spend the vast majority of the time at or near one or other of the two density maxima between the walls, transferring from one extremum to the other at high speed. At any time, only about ten per cent of the atoms will be in intermediate positions; in other words, the transition time is about a tenth of the average time spent at one of the extrema.

So much has become apparent largely because the numerical calculations consist of iterations of the state of the system at intervals of time that are extremely short compared with the physical characteristics of the system. The basic time-step used, for example, is equivalent to one twentieth of the velocity auto-correlation time, which is an inverse measure of the time over which velocities persist unchanged. A coarser timestep might well have obscured this fine detail.

In itself, this says little for the behaviour of real fluids tightly constrained by external boundaries except that they are unlikely to behave as fluids in the directions in which they are constrained, whatever their properties in perpendicular directions. On the face of things, the transverse behaviour of the fluid involving the hopping of atoms from one favoured position to another may be a sign that the fluid behaves as a liquid in that direction and as a gas in the two other directions.

It would be interesting to see what would happen to this model (as to other models) if the basic parameters were different, and in particular if they were such as to make condensation probable and recognizable (which is not that simple in such a narrow slab of fluid). Whether it would be possible to extend the calculations to atoms with directional preferences, as might be expected of water molecules, is more problematical. Already, the authors note that each time-step of their calculation takes 1.8 seconds of time on an array of 17 parallel-wired transputers — the British-made microprocessors that come nearest to being computers on a single chip. That seems to imply a few days for each simulation.

But Powles, Dornford-Smith and Evans seem most of all intrigued by having stumbled on a neat way of testing whether experimental data fit Poisson statistics. Modishly, they refer to an article dating from 1910 in which Rutherford, Geiger and Bateman regret the difficulty of demonstrating the Poisson nature of radioactive decay by accumulating data about the intervals of time between successive decay-events (*Phil. Mag.* **20**, 698; 1910), chiefly for lack of data-handling techniques.

The case of the constrained fluid is more easily handled. If an event is the crossing of an atom from one extremum to the other, the intervals, t, between events are readily calculated and recorded, from which it is possible to work out all possible quantities such as $\langle t^n \rangle$, where n is an integer and the angle-brackets mean averages. But the outstanding characteristic of a Poisson distribution is that $\langle t^n \rangle / \langle t \rangle^n = n!$, whatever the particular parameters defining the distribution. So the constrained fluid is christened a "liquid Poisson oscillator". The name is inconsequential, but the model is not. **John Maddox**

EUKARYOTE ORIGINS

Seeds of a universal tree

David Penny and Charles J. O'Kelly

ACCORDING to the serial endosymbiosis theory, mitochondria and chloroplasts arose by intracellular symbioses between ancestral eukaryotes and free-living unicellular organisms. For these organelles the principle is now well established. But are they monophyletic or polyphyletic, and if polyphyletic, from how many independent ancestral lines do they derive? On page 148 of this issue[1], Douglas *et al.* now report on an analysis of small-subunit (18S) ribosomal RNA sequences from a cryptomonad flagellate (*Cryptomonas* Φ) which shows that chloroplasts have polyphyletic origins, and that some eukaryotes obtained their chloroplasts from other eukaryotes.

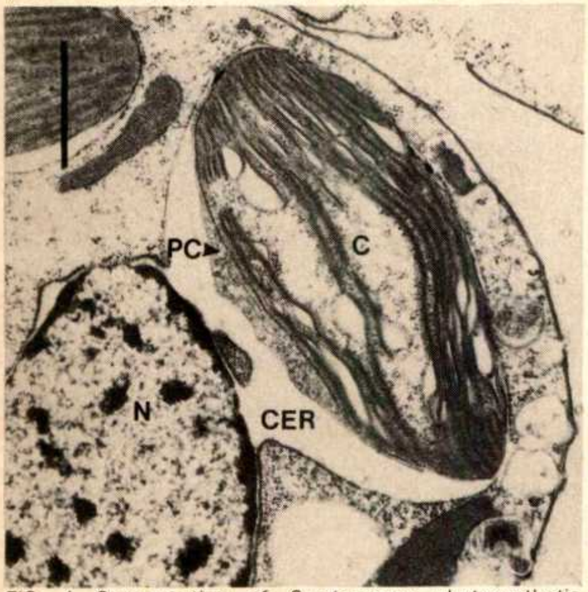


FIG. 1 Cross-section of *Cryptomonas* photosynthetic compartment showing the chloroplast endoplasmic reticulum (CER). The CER contains the nucleomorph (NM), chloroplast (C) and periplastidial cytoplasm (PC). The outer membrane of the CER is continuous with the nuclear envelope and is studded with ribosomes. N, nucleus. Chromophytes have a similar CER but lack the nucleomorph and periplastidial cytoplasm. Chlorarachniophytes possess the nucleomorph and periplastidial cytoplasm, but the outer 'CER' membrane lacks ribosomes and continuity with the nuclear envelope. Scale bar, 1.0μm.

Why study cryptomonads? This aptly named group of phytoflagellates has a perplexing assortment of structural and biochemical features[2], rather as if they had browsed among the other groups of protists and picked out the features that they liked. Amid the confusion, one structure is particularly notable: the nucleomorph. This organelle, bound by a double membrane, looks like a nucleus on a diet and contains both DNA and RNA. What is more, together with the chloroplast(s) and associated cytoplasm, the nucleomorph is enclosed within the 'chloroplast endoplasmic reticulum' membrane system (Figs 1 and 2). Even when the nucleomorph was first described some 25 years ago, it was recognized that the organelles within this membrane system could be the remains of an endosymbiotic eukaryote, with the nucleomorph being its vestigial nucleus. If this were true, then *Cryptomonas* would be expected to have two sets of eukaryotic ribosomal sequences.

From total *Cryptomonas* DNA Douglas *et al.*[1] were indeed able to isolate and sequence two functional 18S RNA molecules, whereas nuclear DNA yielded only one of them. The inference that the other is from the nucleomorph is reasonable. The sequences were analysed by distance matrix and maximum parsimony methods and added to the now familiar, ever-growing universal evolutionary tree.

It is always important to estimate the reliability of an evolutionary tree. To do this, Douglas *et al.* used bootstrap resampling methods to generate new sets of data and inferred evolutionary trees from each set. The resulting trees are compared to find the most stable branches on the original tree. Bootstrapping is now a minimal requirement for an evolutionary study. In the present case, assignments within some major eukaryote clades seem to be robust although relationships between major clades are not. But importantly, the cryptomonad nucleus and nucleomorph appear on different places on the tree and their relative separation seems to be robust. The nuclear rRNA is most closely related to that of fungi, *Acanthamoeba* and green plants, whereas the nucleomorph rRNA segregates with nuclear rRNAs from red algae.

The phylogenetic position of the nucleomorph sequence is supported by other molecular data. For example, both large and small subunits of the photosynthetic enzyme ribulose bisphosphate carboxylase are encoded on cryptomonad chloroplast DNA[3]; only the large subunit is encoded on the chloroplast DNA of plastids containing chlorophyll *b*. In addition, sequences of both subunits of this enzyme in *Cryptomonas* Φ plastids are most closely related to those of red algae. Indeed, the cryptomonad and red algal sequences form a clade separate from the one that includes cyanobacteria, cyanelles and green plants[3,4]. Both the rRNA and ribulose bisphosphate carboxylase gene sequences indicate separate origins at least for 'green' and 'red' plastids, and that red plastids have been secondarily acquired by cryptomonads.

Another important conclusion comes from comparing *Cryptomonas* nuclear sequences with those of other eukaryotes. Cavalier-Smith[5] postulated that cryptomonads belong to a monophyletic kingdom Chromista, the plastid-bearing members of which have a chloroplast endoplasmic reticulum membrane system. On this model the chloroplasts of cryptomonads arose from a single endosymbiotic event in the ancestral 'eochromist'. Accordingly, nuclear rRNA sequences from cryptomonads and chromists should occur together on the tree. But Douglas *et al.*[1] find that the *Cryptomonas* nuclear sequence is separated by a considerable phylogenetic distance from chromist sequences. Thus the rRNA sequences indicate that chloroplasts in these two groups have arisen from separate endosymbiotic events.

These bits of molecular evidence support predictions, based initially on structural and biochemical studies, that chloroplasts have arisen many times. How do we explain this result? Proponents of chloroplast polyphyly have drawn most of their evidence from observations on plastid diversity, and their ideas are, in essence, *ad hoc* explanations. On the other hand, proponents of monophyly have argued on the basis of the mechanisms involved in the transformation of an endosymbiont into an organelle. This transformation requires many steps, each apparently improbable. For example, it is improbable that when a chloroplast gene is transferred to the nucleus, its protein product will have the correct signals to be transported back into the chloroplast. Cavalier-Smith has therefore argued, logically and consistently, for phylogenies that minimize the number of endosymbiotic events.

The past should not be 'explained' in terms of highly improbable events. But as more data became available the predictions of monophyly, starting from no endosymbiotic events, have had to be modified. How has the apparently impeccable reasoning of the proponents of monophyly misled us?

Part of the answer may lie in mechanisms of transformation still to be discovered. For example, transport of phycoerythrin across thylakoid membranes in *Cryptomonas* Φ does not seem to involve a normal signal peptide[6]. But the answer must also partly be in the other side of the equation — the many opportunities for organelles to form during

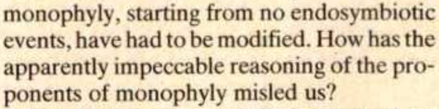


FIG 2. The *Cryptomonas* nucleomorph (NM) with double membrane and nucleolus-like body (asterisk) lies within the CER next to the chloroplast (C). PC, periplastidial cytoplasm. The nucleomorph is about 1μm in diameter at its widest point and is distinctly flattened; the nucleus is 3–5 μm in diameter at its widest point and is more nearly spherical. Scale bar, 0.5μm. Micrographs by C. J. O. and DSIR Electron Microscopy Unit (Palmerston North).

the lengthy course of eukaryote evolutionary history. An analogy is the recent observation[7] that although only one random RNA molecule in 10^{10} may bind a specific dye, if a mole of random RNA molecules (roughly 6×10^{23} molecules) is made, then this vanishingly rare event becomes a certainty.

Let us assume that the same ecological principles applied to the early eukaryotes as apply today. If so, most early photosynthetic organisms would have been protected by a multitude of endo- and exo-symbiotic arrangements[8]. Assume on average that there was the equivalent of a single layer of photosynthetic symbionts (a 'symbiont area index' of one) over the surface of the earth (510×10^3 km^2), each with a surface area of 10 µm^2. This gives the approximate number of photosynthetic genomes as about 10^{20}. If each divided ten times a year then after 100 million years 10^{29} divisions would have occurred, each offering an opportunity for gene transfer between symbiont and host. If anything, this crude calculation is an underestimate of the number of genomes available for transformation into plastids. Success may be rare but opportunity is great.

It would be a mistake, though, to believe that we have done more than begin a proper inquiry into organelle polyphyly and the origin and early diversification of eukaryotes. In much of the present work, too much reliance is placed on results from a single gene, and too much other information is ignored. Study of groups, such as mammals, that diverged much more recently than protists indicates that it is necessary to combine the analyses of several sequences to get results with any sort of reliability[9]. It would be surprising to find that a single gene, even a long one, were sufficient to infer robust trees for divergences that occurred a billion or more years ago. Even in single-gene sequence analyses, additional data are often needed before the trees can be considered to be reliable, as the bootstrapping results by Douglas *et al.*[1] indicate for 18S rRNA trees.

As a result, several fundamental questions remain. At least some chromist chloroplasts, according to sequence analysis of ribulose bisphosphate carboxylase genes, belong in the same clade as cryptophyte and red algal chloroplasts[3]. The resulting dilemma seems to be best resolved by proposing that the endosymbionts separately obtained by cryptophytes and chromists were closely related red algae. But will this explanation survive more rigorous analysis? Differing G+C nucleotide contents seem to confound the relationship between prochlorophytes and green plant plastids[8] and analyses will have to adjust for this. More rigorous analysis would also help confirm that green plant plastids are related to glaucocystophyte cyanelles but originated separately[3].

The most immediate progress in this research area will probably come from studies designed around a specific evolutionary question, often suggested from the literature of protist structure and biochemistry, and will involve thorough analysis of not one but several molecular sequences and checking of the results against theories generated by the non-molecular disciplines. The body of work that is accumulating on *Cryptomonas* Φhas many of the required attributes, and suggests that the simplistic first stage of the search for the single universal evolutionary tree is coming to an end. ☐

David Penny and Charles J. O'Kelly are in the Department of Botany and Zoology, Massey University, Palmerston North, New Zealand.

---

1. Douglas, S.E., Murphy, C.A., Spencer, D.F. & Gray, M.W. *Nature* **350**, 148–151 (1991).
2. Gillott, M. in *Handbook of Protoctista* (ed. Margulis, L., Corliss, J.O., Melkonian, M. & Chapman, D.J.) 139–151 (Jones & Bartlett, Boston, 1990).
3. Valentin, K. & Zetsche, K. *Pl. molec. biol.* **15**, 575–584 (1990).
4. Douglas, S.E. Durnford, D.G. & Morden, C.W. *J.Phycol.* **26**, 500–508 (1990).
5. Cavalier-Smith, T. *Prog.phycol. Res.* **4**, 309–347 (1986).
6. Reith, M. & Douglas, S. *Pl. molec. Biol.* **15**, 585–592 (1990).
7. Ellington, A.D. & Szostak, J.W. *Nature* **346**, 818 (1990).
8. Lockhart, P. thesis, Univ. Sydney (1990).
9. Penny, D., Hendy, M.D., Zimmer, E.A. & Hamby, R.K. *Aust.syst. Bot.* **3**, 21–38 (1990).

MOLECULAR EVOLUTION

The resistance movement

J. F. Y. Brookfield

ATTEMPTS to reduce the incidence in developing countries of diseases transmitted by insect vectors have frequently been hampered by the evolution of insecticide resistance in the vector species. One of the best examples of this problem comes from the efforts to quell the spread of disease by mosquitoes. Mosquitoes of the *Culex pipiens* complex often become resistant to organophosphorus insecticides. Resistance arises from the overproduction of nonspecific esterase enzymes which is usually mediated by the amplification of the corresponding structural genes[1].

Raymond *et al.* now report on page 151 of this issue[2] that the restriction maps of the amplified B2 alleles of the esterase gene in mosquitoes from around the world are indistinguishable. The authors conclude that the amplified B2 genes probably all originated from an initial amplification event that spread rapidly throughout the world by migration. As well as having implications for those interested in designing insecticides against mosquitoes, the new finding shows just how informative DNA sequences are in reconstructing the evolutionary history of alleles within populations.

The allelic esterase alleles B1 and B2 are often amplified as many as 250 and 60 times respectively in mosquito strains which overproduce esterase. Raymond *et al.*[2] analysed the restriction maps of amplified B2 esterase genes in strains from Texas, California, Pakistan, the Ivory Coast, the Congo and Egypt, and found that the maps were the same for the 24 restriction sites at this locus. An experiment of this kind not only looks directly at the 144 bases found at the restriction sites, but also looks indirectly at other six-base sequences that differ by a single base substitution from a restriction site. Furthermore, the authors found that the sequences do not differ by any insertions or deletions of DNA which are frequently found among flanking sequences of *Drosophila* alleles[3]. Although it is impossible to calculate precisely the statistical strength of the inference, the results imply that all these amplified alleles had a recent common ancestor. The most likely explanation is that a single B2 allele was amplified and that the resulting amplified array spread throughout the world owing to the selective advantage that it confers under insecticide treatment.

The result is of great significance to those interested in designing schemes for maximizing the effectiveness of insecticides used against mosquito populations. If insecticide-resistance alleles can become ubiquitous throughout the world by migration from where they initially arise, rather than through their independent occurrence in different places, then other resistance alleles found worldwide could result from rare events in the insect genome. Small-scale trials of new insecticides over limited areas may thus compromise their subsequent large-scale use, because new resistance alleles selected in small areas may quickly spread throughout the range of the species.

The work also has a more general importance for those interested in adaptive changes in wild populations. First, it shows that there can be a remarkably rapid spread of a new allele across continents in mosquitoes, probably by the unintentional help of humans[4]. Second, it illustrates another way in which the information content of DNA can be used to reconstruct the history of changes in allelic frequencies in wild populations. There is a long history of study of genetic polymorphisms defined by charge differences in soluble enzymes[5]. Attempts to understand the selective forces, if any, acting on such polymorphisms have often been confounded by the possibility of heterogeneity existing among alleles belonging to the same electrophoretic class. Two alleles indistinguishable by the electrophoresis of their products may not be identical at the DNA level and thus may not necessarily be subject to the same selection[6]. Nor would their apparent identity necessarily result from shared descent.

The analysis of DNA sequences, however, has held out much hope for those wishing to discover the evolutionary history of alleles, partly because of the high information content of DNA sequences. In *Drosophila*, where many alleles have been surveyed, the heterozygosity at the base-pair level is, as would be expected, variable between loci; and estimates based on restriction-map variation generally fall in the range from 0.2 to 1.2 per cent[7–9]. This means that even at the level of restriction mapping, large numbers of distinguishable alleles exist at a typical locus, and with the use of the polymerase chain reaction to amplify and sequence stretches of DNA yet more allelic variants will be detected.

The other advantage that DNA sequences offer in tracing the evolutionary history of alleles is that most base differences between alleles probably have very little effect on the phenotype. Thus most changes in DNA sequence do not result from natural selection, but occur randomly. As a result, there is little chance of such changes being convergent. It is this assumption that allows Raymond *et al.* to conclude from the identity of their restriction maps that the amplified B2 alleles arise from recent shared descent.

Studies of DNA sequences may thus considerably increase the precision with which the evolutionary history of genes within populations can be reconstructed. It has long been known that the DNA sequences of a particular gene from different species can be used to construct an estimate of the phylogeny of the alleles, and thus of the species possessing them. Similarly, the genetic variation between alleles within species is so great that it is possible to study the phylogeny of the alleles within a population. This phylogeny is, itself, the consequence of the selection and genetic drift that have occurred at the locus. And in turn, comparison of the allelic phylogeny with expectations based on the neutral theory can indicate that selection has taken place.

The phylogeny of a collection of alleles from a population is not a well-defined concept, of course, because recombination causes such phylogenies to vary between different parts of a gene. This, indeed, forms the basis of the power of the technique, as it is the differences between the phylogenies connecting the alleles of different parts of a gene that give clues as to the nature of the selection operating. Thus because vertebrate mitochondrial DNAs do not recombine, the whole molecule must share the same evolutionary history. Consequently, if selection has played a part in the determination of the phylogeny of mitochondrial DNA, there is no way of knowing which of its genes have been of particular selective importance. ☐

J. F. Y. Brookfield is in the Department of Genetics, School of Biological Sciences, University of Nottingham, Nottingham NG7 2UH, UK.

---

1. Mouches, C. *et al.* Science **233**, 778–780 (1986).
2. Raymond, M., Callaghan, A., Fort, P. & Pasteur, N. *Nature* **350**, 151–153 (1991).
3. Aquadro, C. F. *et al.* Genetics **114**, 1165–1190 (1986).
4. Andreadis, T. G. *Am. Mosq. Control Ass.* **4**, 256 (1988).
5. Selander, in *Molecular Evolution* (ed. F. J. Ayala) 21–45 (Sinauer, Sunderland, 1976).
6. Coyne, J. A., Felton, A. A. & Lewontin, R. C. *Proc. natn. Acad. Sci. U.S.A.* **75**, 5090–5093 (1978).
7. Eanes, W. F., Labate, J. & Ajioka, J. W. *Molec. Biol. Evol.* **6**, 492–502 (1989).
8. Langley, C. H. & Aquadro, C. F. *Molec. Biol. Evol.* **4**, 651–663 (1987).
9. Leigh Brown, A. J. *Proc. natn. Acad. Sci. U.S.A.* **80**, 5350–5354 (1983).

BALL LIGHTNING

Great balls of fire

Stanley Singer

KAPITZA proposed in a classic paper that ball lightning is formed by short radiowaves generated in natural atmospheric processes[1]. Now, extending experimental studies by previous investigators of radiofrequency discharges, Ohtsuki and Ofuruton, who report on page 139 of this issue[2], have produced long-lived fireballs in air. The motion of the discharges against the flow of gas and their apparent penetration through a dielectric surface are of particular interest, having been among the most difficult to explain of the properties observed in natural ball lightning.

Mary Evans

Caught unawares — ball lightning at Gorges de Loup.

Ball lightning has been the subject of investigation by distinguished figures in science since the early nineteenth century. Although its appearance is very haphazard and far less frequent than ordinary linear lightning, it is a source of astonishment when the ball of fire is observed in the lower atmosphere, often entering dwellings while floating at a leisurely pace. By its random appearances, it has eluded measurement with the scientific instruments which have provided so much information about ordinary lightning. Even photographs are rare. However, from the qualitative reports of eye-witnesses (now numbering in the thousands, particularly from collections from the Soviet Union) the general properties can be deduced despite the wide variability found in the reports.

Scepticism as to the actual existence of ball lightning has occasionally been based on the assertion that no qualified scientist has ever observed the fireball. The assertion has been incorrect for almost a century. More recently in addition, R. C. Jennison, the noted radioastronomer, and, indeed, Ohtsuki himself have been inadvertent observers of the mysterious fireball; and B. Pippard reported its appearance at the Cavendish Laboratory[3].

Ball lightning makes its appearance as a flaming globe, typically orange, red–orange or intense white, less often blue, green or yellow. The ball is usually 25–30 cm in diameter, with much smaller and much larger dimensions noted in individual cases (from 1 cm to 10 m and larger). Occasionally, the balls move against the direction of prevailing winds and penetrate window panes without making a hole in the glass; it is these properties that Ohtsuki and Ofuruton now report from their experimental discharges. An absence of warmth has been noted in some incidents in which the ball passed particularly close to an observer; in others the witness was burned, or combustible materials were ignited. Occasionally the globe disappears with a frightful explosion.

There is an even greater disparity in the reported conditions for the appearance of ball lightning. For a century and a half, the observations in Europe and the New World were almost invariably associated with thunderstorms and often directly with the occurrence of ordinary lightning. In several cases ball lightning has been seen at the tip of a zig-zag lightning flash or alongside the bright channel of the discharge. There were only a few reports of ball lightning appearing in fair weather or in the absence of a storm. A recent collection of hundreds of observations in Japan, however, contains ten times as many cases in fair weather as in rain or storm[4]. These data render theoretical descriptions of generation of the fireballs from lightning channels somewhat less convincing, at least as the only possible mechanism.

The difficult and long-standing problem of ball lightning has attracted few meteorologists or atmospheric scientists. Rather, physicists constitute the larger part of the company studying the atmospheric fireballs. Numerous theories have been put forward, covering chemical, electrical, nuclear and relativistic models. New states of matter have been proposed to explain the unusual properties reported for ball lightning. Often specialists conclude that solutions must be found outside their own field. Thus, Faraday commented that ball lightning cannot be related to lightning or to any form of electrical discharge because the discharges he was familiar with, exist only briefly and travel at

---

1. Kapitza, P. L. *Dokl. Akad. Nauk SSSR* **101**, 245 (1955).
2. Ohtsuki, Y. H. Ofuruton, H. *Nature* **350**, 139–141 (1991).
3. Pippard, B. *Nature* **298**, 702 (1982).
4. Ohtsuki, Y. H. *Baryon* **1** (12), 96–97 (1986).
5. Smirnov, B. M. *Phys. Rep.* **188**, 1–78 (1990).
6. Zheng, X-H. *Phys. Lett.* A**148**, 463–469 (1990).
7. Muldrew, D. B. *Geophys. Res. Lett.* **17**, 2277 (1990).
8. Lushnikov, A. A., Negin, A. E. & Pakhomov, A. V. *Chem. Phys. Lett.* **175**, 138–142 (1990).
9. Cawood, W. & Patterson, H. S. *Nature* **128**, 150 (1931).

high velocity. Similarly, the plasma physicists B. M. Smirnov and E. Witalis have concluded independently that ball lightning cannot be a plasmoid. Smirnov is presently considering fractal combustion models with an aerogel structure[5].

Three types of fireball have been noted in laboratory experiments having qualitative resemblance to ball lightning. These laboratory fireballs are the globes formed in dilute mixtures of fuel gas in air, in some radiofrequency gas discharges and luminous spheres produced from metal wires by electric discharge. None of these is yet adequately understood. All show the possibility of a lifetime sufficient to account for that of ball lightning and, in some cases, have additional properties associated with ball lightning. In previous work, Ohtsuki and Ofuruton studied the ignition of dilute fuel gases. They failed to duplicate the previous results of investigators who produced long-lived fireballs in homogeneous gas mixtures. They found it necessary to add combustible aerosol particles for the formation of fireballs.

Now Ohtsuki and Ofuruton have turned their attention to radiofrequency discharges, as reported in this issue. The new results confirm the long lifetimes observed in previous studies. But more important is the observed motion of the fireball against the flow of air in their system (confirming one of Kapitza's predictions) and passage without damage through a ceramic surface placed in the experimental system. In reproducing these most unusual aspects of ball lightning, the new work represents a considerable advance over previous studies on radiofrequency discharges, including one by Tesla, which were largely limited to describing qualitative properties of lifetime, shape and colour.

In other recent theoretical work, analyses have been made of spherical fireballs containing internal electromagnetic radiation trapped by a surface plasma layer[6,7]. The trapped radiation provides the continuing energy needed to maintain the sphere for the observed long lifetime. This mechanism may be related to Kapitza's theory if the plasma ball is permitted to trap some of the radiation that leads to its formation, rather than expelling it. Also, an experimental paper[8] showed that a filamentary aerogel can form in a strong electric field through the aggregation of aerosol particles. This process was anticipated in the work of Cawood and Patterson[9] on the electrostatic coagulation of small dye crystals suspended in air. Very demanding experimental conditions are needed for the laboratory synthesis of aerogels which have been suggested as a structure of ball lightning. ☐

Stanley Singer is at Athenex Research Associates, 381 South Meridith Avenue, Pasadena, California 91106, USA.

Mary Evans

Silent, vanishing balls of fire at Agé, near St Petersburg, 1888.

PHOTOSYNTHESIS

Absorbing developments

M. C. W. Evans and J. H. A. Nugent

Photosynthetic organisms have two tasks in the early stages of photosynthesis. One is to operate an efficient process whereby solar energy is converted to chemical energy; the other is to capture photons with a high efficiency and at reasonable metabolic cost. Energy conversion is carried out by photosynthetic reaction centres, in which the initial solid-state photochemical reactions take place in picoseconds, and knowledge of the mechanisms involved has advanced rapidly as the result of a combination of spectroscopic and crystallographic work on the purple bacterial reaction centre[1,2]. Our understanding of light harvesting is less good. But as Kühlbrandt and Wang demonstrate on page 130 of this issue[3], here too rapid advances are being made using the electron crystallography techniques developed by Henderson with bacteriorhodopsin[4]. This technique allows detailed structures to be developed from the two-dimensional crystal arrays available for these proteins and has now resulted in the determination, to 6Å resolution, of the structure of a pea light-harvesting complex.

```
Helix A
              56                                  91
               - -   + +- -  + +            -   +
               DPETFAKNRELEVIHSRWAMLGALGCVFPELLSRNG
                ............:::::===========:::::.

Helix B
             170                                   206
               - -   - + +- +  +                 +
               DPEAFAELKVKELKNGRLAMFSMFGFFVQAIVTGKGP
                ..............::::================:.

Helix C

                  117                      143
                      +                  -  +
                  PSLVHAQSILAIWATQVILMGAVEGYR
                     ::=================::
```

Amino-acid sequences (single-letter code) of the transmembrane helices of a pea light-harvesting complex. Helices are labelled A, B, and C, as by Kühlbrandt and Wang[3]. The hydrophobic core of the membrane is approximately 30Å or 20 helical amino-acids wide. Structure prediction indicates that helices A and B extend beyond the membrane and that the lipid phase may contain charged amino acids. The uncharged core of the predicted membrane span is shown as [=], residues near the edge of the membrane span as [:] and the possible helical extension at the N termini of helices A and B as [.] Helix C forms a 'classic' transmembrane helix with a stretch of about 20 uncharged amino acids spanning the lipid bilayer.

Reaction centres and their associated electron-transport chains are complex structures that are metabolically expensive to make. The photochemical energy-conserving reaction takes place in a pair of chlorophyll molecules in a special environment. But even in bright sunlight individual chlorophyll molecules will be hit by a photon only about once a second. The problem of providing effective light absorption and rates of energy transfer to the reaction centre at low metabolic cost has been overcome by surrounding the reaction centre by a bed of pigment molecules — the light-harvesting or antenna complex — which must be organized to provide efficient energy transfer. Following the absorption of a photon, the excited state generated can migrate to other chlorophylls very rapidly by one of two routes (delocalized exciton coupling or Förster transfer). The excitation follows a random path through the pigment bed but is effectively spiralling down an energy gradient to reach the chlorophyll in the reaction centre, which absorbs longer wavelengths and therefore lower energies. In practice, plant reaction centres may therefore turnover more than 100 times per second and operate at maximum efficiencies at low light intensities.

The structure and efficiency of the pigment bed supplying the reaction centre must be optimized by specific positioning of pigments within pigment protein complexes of the photosynthetic membrane. In higher plants the main components of the pigment bed are the chlorophyll *a*/*b* binding (CAB) proteins. From secondary structure analysis CAB polypeptides are predicted to have two to four membrane-spanning helices, although a three-helix model is widely accepted[5]. Kühlbrandt and Wang identify three helices. But this highlights a problem with some of the putative membrane-spanning helices in CAB proteins, namely that it is impossible to model three helices without including charged residues in the membrane (see figure). The CAB helices are also interesting in that two are considerably longer than expected or required simply to cross the hydrophobic part of the membrane, and are longer than those found in the reaction centre or bacteriorhodopsin, which may indicate some anchoring or binding function outside the membrane.

Another question which must be answered for light-harvesting complexes has to do with where the chlorophylls are bound. Crystals of bacterial light-harvesting proteins contain only 18 chlorophylls in an oligomer of relative molecular mass 84,000 (M_r 84K)[6], whereas the CAB proteins contain 15 in a polypeptide of M_r only 25K. Chlorophyll appears to be associated with specific residues within reaction centres, the 'traditional' residues being histidine, glutamine and asparagine. But there are not enough highly conserved residues among the family of CAB proteins to bind the associated chlorophylls. Unless there are specific residues in each type of CAB, this implies that there is some other form of liganding through water molecules of backbone carbonyls. In the structure determined by Kühlbrandt and Wang, the chlorophylls are arranged on the outside of the protein, which may perhaps facilitate energy transfer to the reaction centre or between CAB molecules. Combination of the results from further refinements of the present structure, with results from X-ray analysis of three-dimensional crystals of the light-harvesting protein from purple photosynthetic bacteria[6], will allow the development of a full model of light-harvesting structures.

As is only to be expected, the partial resolution of a protein structure poses many more questions than it answers. Inevitably we must ask for even greater resolution and detail, and we need to distinguish the chlorophyll *a* and *b* molecules and define their absorption wavelengths. From the present structure it would seem that there is heterogeneity between the parts of the protein in each leaflet of the lipid bilayer. Does this mean that there is a particular pathway in or out of the monomeric complex to other similar complexes or to the reaction centre? Each plant species contains a number of CAB proteins, and although the structure determined by Kühlbrandt and Wang seems to offer a general model there must be small but important differences between them. The most important question will be how these proteins interact with the reaction centre to provide effective energy transfer. ☐

M. C. W. Evans and J. H. A. Nugent are in the Department of Biology, University College London, Gower Street, London WC1E 6BT, UK.

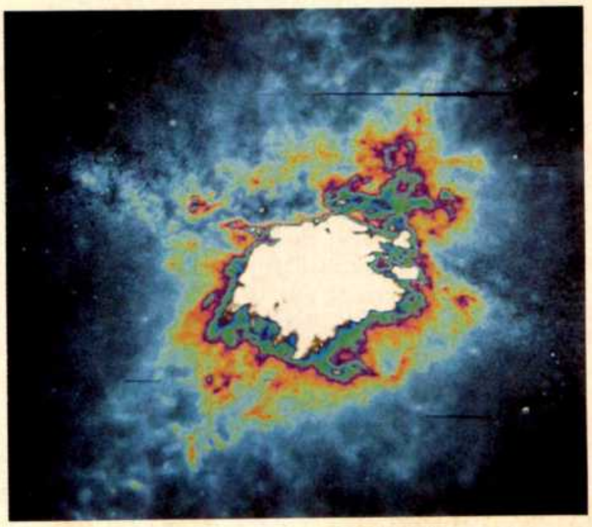
1. Deisenhofer, J., Epp, O., Miki, K., Huber, R. & Michel, H. *Nature* **318**, 618–624 (1985).
2. Feher, G., Allen, J.P., Okamura, M.Y. & Rees, D.C. (1989) *Nature* **339**, 111–116 (1989).
3. Kühlbrandt, W. & Wang, D.N. *Nature* **350**, 130–134 (1991).
4. Henderson, R. *et al. J. molec. Biol.* **213**, 899–929 (1990).
5. Karlin-Neumann, G.A., Kohorn, B.D., Thornber, J.P. & Tobin, E.M. *J. molec. appl. Gen.* **3**, 45–61 (1985).
6. Papiz, M.Z. *et al. J. molec. Biol.* **209**, 833–835 (1989).

MILKY WAY

Not with a whimper

Leo Blitz

The centre of the Milky Way appears to be primed to explode in a profusion of star formations leading to the explusion of vast amounts of matter from our Galaxy. This is the conclusion of A. Stark and colleagues, based on an analysis of radioastronomical observations of the central regions of the Milky Way and reported in the *Monthly Notices of the Royal Astronomical Society* (**248**, 14p – 17p; 1991). This starburst will occur 10–100 million years hence, they suggest, a time very short compared to the age of either the Milky Way or the Solar System.



The prototypical starburst galaxy, M82. The image shows an extensive system of filaments extending several kiloparsecs above and below the starbursting disk. The kinematics and other physical properties show that gas is flowing out in a large-scale bipolar patter, probably driven by the starburst. The colours in this false-colour image indicate the intensity of line emission (Balmer–α) from atomic hydrogen. (Courtesy of T. Heckman and L. Armus.)

The Milky Way has been known for some time to harbour massive clouds of gas and dust in its central regions. Similar but smaller clouds occur throughout the disk of the Galaxy, and are the material from which new stars form. Known as giant molecular clouds, these objects are composed primarily of molecular rather than atomic hydrogen, and are typically a hundred to a thousand times denser than the mean for interstellar gas. In our part of the Galaxy, about half way out in the disk, giant molecular clouds typically have enough material to form about 100,000 stars with a mass like that of the Sun. The efficiency is known to be low, however, and only a few per cent of the gas is actually turned into stars.

Occasionally, a few stars form, known as O stars, that are so bright that the radiation from their surfaces accelerates a wind of ions at velocities up to about 1 per cent of the speed of light. The Sun also generates a wind of ions, but at rate as much as 10^{10} times weaker than the O star. The ultraviolet radiation from the O stars is so intense that it destroys (by dissociation and ionization) the molecular clouds out of which they are born in a sort of cosmic matricide. Finally spent in the relatively short time of 3 million years, an O star suffers a cataclysmic explosion visible as a supernova which leaves a remnant neutron star or black hole.

In our region of the Milky Way, giant molecular clouds typically form fewer than 10 O stars at any one time, and although the phenomena associated with these stars are quite spectacular, their small numbers ensure that their effects are limited to a relatively small volume near the stars themselves. In the centres of some galaxies, however, the star formation is so vigorous that as many as a million O stars are formed almost simultaneously. The collective effects of these stars can produce a brilliant galactic nucleus and give rise to spectacular ejections of matter from stellar winds and supernova explosions. These rare galaxies, known as starburst galaxies, contain unusually large concentrations of molecular gas at their centre. The nearest example of a starburst, shown in the photograph, is the galaxy M82. The bright nucleus is hidden by the intervening dust, but the disturbed appearance testifies to the enormously energetic activity going on at the centre. In spite of its spectacular appearance, as starbursts go, M82 is rather wimpy.

It has been known since the 1970s that the nucleus of the Milky Way contains a vast reservoir of molecular gas — enough to make more than 10 million O stars if all of the gas could be converted this way. But until recently, there was no reason for supposing that this gas could not maintain itself in stable orbits around the centre, or at worst spiral slowly and harmlessly into the centre owing to the viscosity of the gas. What Stark *et al.* have shown is that the molecular gas that is collected into the largest discrete clouds, which are a substantial fraction of the total, will inevitably spiral into the nucleus in a time no more than about 1 per cent of the age of the Milky Way, and perhaps considerably less.

The reason for this is dynamical friction, a gravitational analogue of what happens if one suddenly shuts off the motor of a boat ploughing through the water. Most of the stars in the nucleus of the Milky Way are part of the central bulge which contains stars rotating relatively slowly about the centre. The molecular clouds in the innermost regions of the Galaxy rotate about the centre much more rapidly. Thus, as the molecular clouds move through the more sluggish stellar system, the stars exert a gravitational drag causing the clouds to give up some of their orbital momentum and consequently fall into the nucleus of the Galaxy. The orbital energy of the clouds is dissipated in stellar wakes which increase the energy of the stars, just as the hull of a boat raises a wake on the surface of the water. As the clouds fall into the centre they will collide and coalesce, forming massive aggregations which are ripe for the formation of the vast number of O stars inferred to be present in starburst nuclei.

It is likely that an effect considered by Stark *et al.*, but not explicitly analysed by them, will cause the infall of the molecular clouds to be even faster than that caused by dynamical friction. There is now direct evidence that the centre of the Milky Way contains a stellar bar, a system of stars agregated in an elongated pattern that rotates about the centre as a solid body. Simulations of gas flow in a barred self-gravitating system suggest that the gas in the bar will fall almost radially towards the centre, shortening by a large margin the amount of time it would take for the gas to get there. However, a detailed calculation for the Milky Way cannot be done until infrared observations of the bar by the Cosmic Background Explorer (COBE) satellite can be interpreted to give a reliable determination of the precise mass distribution of the bar. So it is yet to be determined whether dynamical friction or a barred distribution of stars plays a more important role in the development of the starburst. The issue is an important one because in the latter case, a disruption of the clouds as a result of star formation prior to reaching the nucleus should be relatively less important than if dynamical friction is the primary mechanism by which angular momentum is lost.

Many details are still to be worked out. We do not yet know what physical conditions are necessary for the molecular gas to form the vast numbers of O stars in a starburst. Furthermore, given the reservoir of molecular material at the Galactic Centre, it is not yet possible to predict whether the Milky Way will be either a macho or wimpy version of a starburst. But the general conclusions of the Stark *et al.* paper seem rather robust. Further work should help clarify whether the Milky Way and normal disk galaxies in general all have the capability of becoming starbursts, implying that the starburst phenomenon is an episodic one, or whether only certain galaxies have the right stuff to become starburst. And what's all that molecular stuff doing in the centre of the Milky Way anyway? ☐

Leo Blitz is in the Astronomy Program, University of Maryland, College Park, Maryland 20742, USA.

BONE MARROW TRANSPLANTATION

Making just the right match

Peter Parham

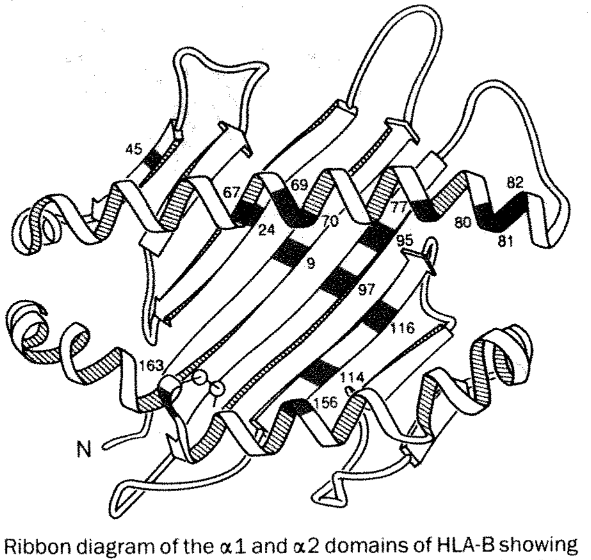
TRANSPLANTATION of organs and tissues between individuals is now a routine treatment for a variety of diseases. A drawback of these therapies is immunological rejection of the graft, a response stimulated by antigenic differences between the class I and II HLA molecules of the donor and the recipient. In practice the gravity of the problem varies with the tissue transplanted. Solid organs — kidney, heart and liver — mismatched at several HLA genes are effectively transplanted under cover of immunosuppressive drugs, anti-T-cell antibodies and lymphoid irradiation, whereas the success of bone marrow transplantation is greatly affected by the quality of the HLA match. The extreme sensitivity of bone marrow transplantation to HLA incompatibility is dramatically demonstrated by a case described by Fleischhaeur and colleagues in *The New England Journal of Medicine*[1], in which rejection was associated with a single amino-acid substitution in a class I HLA molecule.

The transplant recipient, who was suffering from chronic myelogenous leukaemia, and the unrelated bone-marrow donor were judged by tissue typing to be HLA identical. This was not the case, however, for electrophoretic comparison of their class I HLA proteins showed the patient to express a more acidic HLA-B44 than the donor. Furthermore, nucleotide sequence comparisons showed the only difference between the two molecules to be at position 156, where aspartic acid in the patient was substituted for leucine in the donor (see figure). Evidence for cell-mediated graft rejection was observed two weeks after transplantation, and *in vitro* cultures started at that time revealed the presence in the patient's blood of cytotoxic T lymphocytes that killed target cells expressing the donor's, but not the patient's, HLA-B44 subtype. Although, as Fleischhauer *et al.* emphasize, "a cause–effect relationship between these cytotoxic T lymphocytes and marrow-graft rejection has not been proved directly" there are reasons, including many precedents from experimental transplantation in laboratory animals, to believe that is the case.

For over 10 years it has been known that serological tissue-typing does not distinguish certain 'subtypes' encoded by closely related class I HLA alleles[2], an extreme example being the HLA-B27 antigen which embraces the products of at least seven different alleles[3]. Such subtypes can readily be distinguished by other assays and in the case of HLA-B44 the two subtypes were first described on the basis of electrophoretic differences and their differential recognition *in vitro* by alloreactive T cells[4]. As more precise molecular methods have been applied to the analysis of HLA genes, the magnitude of the discrepancy between serological definition and molecular reality has increased[5] — even with the most conservative estimation one must now expect the actual number of class I HLA alleles to exceed the currently defined antigens by a factor of two to three.

The similarity in structure of class I subtypes has led some to suggest that their differences will be of little consequence for transplantation and can therefore be ignored. This assumption works when donors and recipients are being matched within families not because subtype differences are inconsequential, but because the task of typing is reduced to tracking the segregation patterns of entire HLA haplotypes. When an HLA-identical family member is not available, however, and the search extends to panels of unrelated donors[6], the assumption breaks down, as demonstrated by Fleischhauer *et al.*[1].

There is indeed good reason to believe that HLA-A,B subtypes differ in ways that efficiently stimulate alloreactive T cells and provoke graft rejection. With few exceptions the differences between subtypes are at sites that determine the interactions of class I molecules with antigenic peptides or T-cell receptors (see



Ribbon diagram of the $\alpha1$ and $\alpha2$ domains of HLA-B showing the positions of residue 156 in the centre of the α helix of the $\alpha2$ domain. Comparison of the sequences of 35 HLA-B alleles[11] shows that the 16 positions with highest amino acid variability, which are shown as shaded blocks in the diagram and include 156, are all at peptide and T cell receptor–interaction residues of the antigen recognition site. The structure is based on that of HLA-A2 (ref.12).

RÉSUMÉ

Solvent solution

In an epidemiological study published in *The Veterinary Record* (**128**, 199–203; 1991), J.W. Wilesmith *et al.* trace the onset of the bovine spongiform encephalopathy (BSE) epidemic in British cattle back to a change in the practice of meat and bone meal production. Around 1980, most British 'rendering' plants stopped using hydrocarbon solvents to separate fat from carcasses. This abrupt change fits in with the supposed transmission to cattle, through their feed, of the infective agent of scrapie (a similar disease afflicting sheep) in the winter of 1981–82. The authors point out that the additive effects of heat treatment and solvent action, or the additional steam heating needed to remove the solvents, may explain the agent's inactivation when solvents are used, and that regional variation in rendering practice could underlie the distribution of BSE cases.

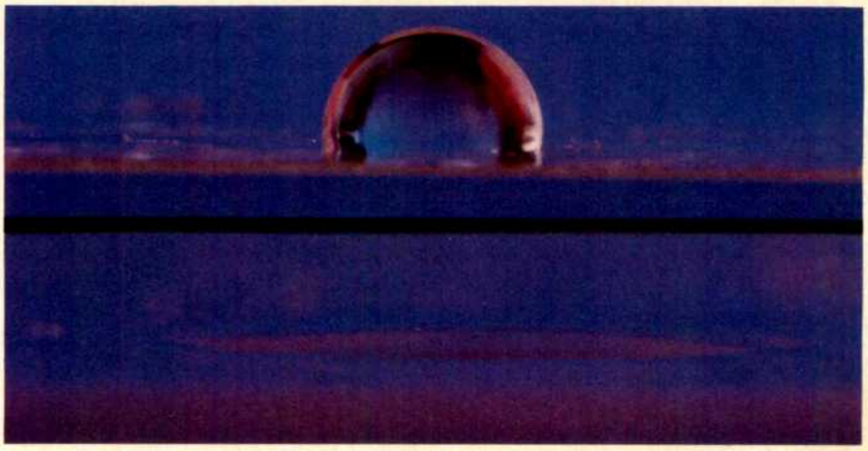
Bated breath

Is the Earth warming up in response to increased atmospheric concentrations of greenhouse gases? The answer, according to T. Karl *et al.* (*Science* **251**, 1058–1061; 1991), is that we won't be able to tell for some time yet. Although the past decade was the warmest on record, it is difficult to distinguish the effects of additional greenhouse gases from natural variations in the climate. Comparing the records of temperature and precipitation for the central United States with model projections for the past 95 years, the authors find no significant indications of warming trends. If the model projections are right, an unambiguous greenhouse signal will emerge only over the next 10–20 years. It will take even longer for any effects of greenhouse warming to show up in levels of precipitation. This leaves policy makers, the authors remark, "in an unenviable position".

Sweet success

Using linkage studies, G.I. Bell and colleagues have mapped the gene responsible for one form of non-insulin-dependent diabetes mellitus (*Proc. natn. Acad. Sci. U.S.A.* **88**, 1484–1488; 1991). First described in the 1970s, maturity-onset diabetes of the young (MODY) is an autosomal-dominant disorder that otherwise closely resembles late-onset non-insulin-dependent diabetes. Using 79 DNA markers, Bell *et al.* excluded about 40 per cent of the human genome, including several candidate genes such as the insulin-responsive glucose transporter on chromosome 17. Close linkage to the MODY locus was finally demonstrated in one large affected family with the adenosine deaminase gene, which maps to the long arm of chromosome 20. Although the location of the MODY gene does not betray its identity, its eventual isolation may provide insight into other forms of diabetes as well.

Fashioning wet-look latex



Whereas water on the surface of most artificial plastics and elastomers beads up as shown in the top frame, Isao Noda has developed (page 143) an elastomeric latex that is fully wettable by water (bottom frame; the fully spread droplet is barely visible). The surface of this polystyrene–polybutadiene latex is rendered hydrophilic by adding a diblock copolymer during synthesis, one end of which is water-soluble (polyethylene oxide, PEO) and the other hydrophobic (ensuring solubility in the hydrocarbon latex). For reasons as yet unclear, the amphiphilic copolymer migrates to the surface during polymerization of the latex constituents, there becoming immobilized with the PEO ends exposed to the air.

Block copolymers, used already as emulsifying agents for polymer alloys, are thus able to influence surface as well as bulk properties. The immediate applications (nappies and bandages) may seem prosaic. But might this be a general way to make surfaces wettable only by specific adsorbates? P.B.

figure). Position 156 at which the two HLA-B44 subtypes differ provides a classic example; it is a peptide-interacting residue, the most variable residue of the $\alpha 2$ domain helix, and substitutions at this position have repeatedly been shown to alter T-cell responses. In the course of human evolution class I subtypes have probably been selected for their capacity to bind and present different disease-related peptides to T cells. Substitutions that endow such properties are likely, given the common mechanism of T-cell recognition, to stimulate distinctive alloreactive cytotoxic T-cell responses. In fact that is precisely how subtypes were discovered in the first place[2]. Thus the experience of Fleischhauer *et al.* with HLA-B44 will probably have general application to cases where transplant donors and recipients are mismatched for class I HLA subtypes.

Another feature of the class I HLA subtypes is their distribution in populations of different geographical origins. For example the B*2703 allele has been found only in African and American blacks, the B*2704 and B*2706 alleles only in orientals, and the B*2701 and B*2702 alleles only in caucasians[3]. These differences, which go undetected by current HLA typing and are likely to provoke allograft rejection, undoubtedly contribute to the difficulty of matching transplant donors and recipients of different racial origins. Such considerations substantiate the view that "The racial differences in ABO blood groups and MHC phenotypes make it more likely that a candidate for transplantation will receive a well-matched kidney from a member of the same race"[7].

Through the use of an ever-expanding arsenal of non-specific immunosuppressants, surgeons have been able increasingly to evade the immunogenetic rules of transplantation. As a consequence, the value of HLA matching for solid organs transplanted between unrelated donors is often called into question. For bone marrow transplantation there is in contrast little doubt as to the importance of obtaining the best HLA match, and over the past 20 years the procedure has evolved into the preferred therapy for a widening range of haematopoietic disease[6,8,9]. This impressive advance has primarily been achieved by the study of bone marrow transplantation between HLA-identical siblings. Now, however,

---

1. Fleischhauer, K., Kernan, N. A., O'Reilly, R. J., Dupont, B. & Yang, S. Y. *New Engl. J. Med.* **323**, 1818–1822 (1990).
2. Biddison, W. E., Ward, F. E., Shearer, G. M. & Shaw, S. *J. Immun.* **124**, 548–552 (1980).
3. López de Castro, J. A. *Immun. Today* **10**, 239–246 (1989).
4. Hahn, A. W. A., Schendel, D. J., Hansen, P. W. & Ploegh, H. L. *Hum. Immun.* **11**, 69–76 (1984).
5. Bodmer J. G., Marsh S. G. E. & Albert, E. *Immun. Today* **11**, 3–10 (1990).
6. Hows, J. M. & Bradley, B. A. *Br. J. Haemat.* **76**, 1–6 (1990).
7. Kasiske, B. L. *et al. New Engl. J. Med.* **324**, 302–307 (1991).
8. Martin, P. J., Hansen, J. A., Storb, R. & Thomas, E. D. *Adv. Immun.* **40**, 379–438 (1987).
9. Santos, G. W. *Clinics Haemat.* **12**, 611–639 (1983).
10. Saiki, R. K., Bugawan, T. L., Horn, G. T., Mullis, K. B. & Erlich, H. A. *Nature* **324**, 163–166 (1986).
11. Bjorkman, P. J. & Parham, P. *A. Rev. Biochem.* **59**, 253–288 (1990).
12. Bjorkman P. J. *et al. Nature* **329**, 506–512 (1987).

most potential marrow recipients in Europe and North America lack an HLA-identical sibling, and this has led to the establishment of large registries of potential donors from which unrelated HLA-matched individuals can be selected[6].

The message from Fleischhauer *et al.* is then that HLA typing and assessment of tissue compatibility for bone marrow transplantation will be of the greatest value if all HLA-A, B and C alleles, subtypes included, can be distinguished by routine tissue typing. Moreover the increasing ethnic diversity of North American and European populations underscores the value of a global approach to defining subtypes. Given the inadequacy of the serological typing methods, meeting these goals will require techniques based upon the nucleotide sequences of the alleles themselves. Such DNA-based typing methods are beginning to be used for class II HLA alleles[10], and the results obtained by Fleischhauer *et al.* must surely catalyse their application to class I. □

Peter Parham is in the Departments of Cell Biology, and Microbiology and Immunology, Stanford University School of Medicine, Stanford, California 94305, USA.

Salvador E. Luria (1912–1991)

S. E. Luria, who died on 6 February, brought bacteria and their viruses, the bacteriophages, into the forefront of research on the nature of the gene.

Born in Turin, Italy, Luria initially trained to be a physician and chose radiology for his speciality. After military service he moved to Rome to learn more physics in the circle of Enrico Fermi and Edoardo Amaldi. There Franco Rosetti excited him about radiation biology and the formulations by the German physicist Max Delbrück of the gene as a molecule. Equally importantly, he discovered the existence of bacteriophages and began experiments on them in the laboratory of the bacteriologist Geo Rio. Growing official anti-semitism in Italy necessitated his move to Paris in late 1938 where he continued working on phage under the patronage of the physicist Fernand Holweck, doing experiments that aimed to determine the sizes of phages through measurements of the rate at which they were inactivated by ionizing radiations.

The German conquest of France forced him again to flee, this time to New York City which he reached in September 1940 by boat from Lisbon. At the suggestion of Fermi, Luria approached the Rockefeller Foundation and the fellowship they provided allowed him to resume phage work at the College of Physicians and Surgeons of Columbia University. Soon he was in contact with Delbrück, also by then a refugee from totalitarian tyranny. By the summer of 1941, they had begun joint experiments on *Escherichia coli* and its phages, first at Cold Spring Harbor and later at Vanderbilt to which Delbrück had moved to teach physics.

Initially, they focused on the interference phenomenon which permits only one type of phage to multiply in a given bacteria. In the course of their experiments, they used bacteria variants resistant to specific phages. At that time it was an open question whether these resistant cells arose through gene mutations, since traditional wisdom among bacteriologists was that bacteria lacked chromosomes and a form of heredity similar to that of higher organisms. In particular the English physical chemist Cyril Hinshelwood argued against mutational origins of resistant bacteria, believing that they arose through altered chemical equilibria.

It was Luria who provided the key idea to distinguish between the mendelian and lamarckian explanations. What was needed was a way to show that the phage-resistant variants of *E. coli* existed before they came into contact with phage. In February 1943, soon after his move to Indiana University, he realized that the distribution of resistant bacteria in a series of different cultures should settle the question. If the bacteria were made resistant by contact with phage, the number of resistant bacteria would be very similar in all the cultures. But if they arose by mutations, they should be clustered in families, the size of which reflected the time at which the mutational events occurred during the growth of the cultures. Depending upon when the mutations took place, there could be one, two, four, eight, and so on, resistant cells in each culture tube.

As soon as Luria found his first evidence favouring gene mutations he wrote to Delbrück, who then worked out the appropriate mathematical equations. The resulting Luria–Delbrück manuscript, which appeared late in 1943, changed the face of genetics by making bacteria the obvious organisms for research on the nature of the gene. Experiments could be done in a day instead of weeks, and billions of cells could be examined in searching for mutants and determining mutation rates.

The following year Luria went on to show that phages also spontaneously mutate as they multiply, giving rise to variants every bit as stable as those found in bacteria. Two years later, Delbrück and Alfred Hershey independently provided evidence for genetic recombination in phage, while Joshua Lederberg and Edward Tatum demonstrated genetic recombination in *E. coli*. Thus, within a brief three-year span, the existence of chromosomes within both *E. coli* and its phages was established. For these experiments and Hershey's subsequent demonstration that DNA was the genetic component of phages, Luria, Delbrück and Hershey in 1969 received the Nobel Prize in Medicine and Physiology.

Less appreciated was the key role played by Luria in the 1952 discovery of the host-modification phenomenon in which the host range of a progeny phage can be influenced by the exact strain of bacterium in which it has multiplied. A decade later, Werner Arber went on to show that such behaviour reflects enzymatic attack on unmodified phage DNA and its prevention by methylation.

Luria was an exceptionally talented writer. His scientific papers, textbooks and books for the general public all reflect mastery of his adopted English language. His first popular book, *Life: The Unfinished Experiment* (1973), won the National Book Award, with his autobiography *A Slot Machine, A Broken Test Tube* (1984) lucidly describing his intellectual and humanistic development. I remember him to be a teacher of the first rank. In autumn 1947, after only a few days into his course on viruses, I wanted to do my PhD under his supervision. It was typical of his devotion to his students' future success that he later arranged with John Kendrew for me to go to the Cavendish Laboratory where I was to meet Francis Crick.

Equally important was his skill as an administrator, shown over the 13 years he wisely and compassionately directed MIT's newly formed Cancer Center.

He was a human of passionate political beliefs. From early youth he identified himself with the causes of organized workers as opposed to those of management. As the Vietnam war developed, he became increasingly prominent in the antiwar movement and refused payment of the war-related component of his income taxes. Oppression of any form disturbed him, and there was never any doubt as to where he stood about authoritarian governments or institutions. More recently he worried about society's handling of the human genetic data whose accretion would be greatly accelerated by the human genome project. The possibility of a genetically defined underclass disturbed him, and we disagreed as to whether our societies would find the means to protect the victims of unjust throws of the genetic dice.

Luria knew what science at its best was and strove at all times to maintain high standards in both science and the human behaviour that make it possible. In doing so while young he could offend out-of-date minds or those who were all too self-satisfied with their accomplishments. By the time he died, however, there were few who did not feel better by being in his presence.

James D. Watson

James D. Watson is at the Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724, USA, and the National Center for Human Genome Research of the National Institutes of Health.

OPTICAL COMMUNICATIONS

As quick as a flash

Paul Calvert

To get the most out of the large capacity of optical fibre communication networks, one wants to be able to switch the signal on and off as fast as possible to transmit the maximum number of noughts and ones. Liquid crystals take several milliseconds to switch, and so are too slow. Lithium niobate is now used and can switch in fractions of a nanosecond. But to get to the desirable switching times of a picosecond or less, one has to rely on the rapid movement of electrons in molecules. Conjugated aromatic molecules offer the most promise for large changes of refractive index, induced by a redistribution of electrons in an electrical or optical field. However, nothing wholly suitable has yet been found. Nothing daunted, C. S. Winter *et al.*, from the British Telecom Research Laboratories, now suggest[1] that nickel dithiolene shows great promise.

As described in a previous News and Views[2], one possible optical switch is a Mach–Zehnder interferometer in which a light signal is split into two: one side travels through an optically active material whose refractive index is modified by an electric field. The increased index slows the light in this arm until it out of phase with the signal in the other arm when they recombine. With the field on, the two arms subtract to give zero; with the field off they add.

Stegeman *et al.*[3] have proposed figures of merit for nonlinear optical materials to balance the efficiency of refractive index change against the loss of signal due to scattering or absorption. Unfortunately the best available material on this basis is silica which has a low nonlinear coefficient but extremely low optical absorption and scattering. A device made from silica would have to be enormously long to get the half-wavelength retardation needed to switch the signal off.

Materials can be made with very large nonlinear coefficients (χ_3) if they have an absorption peak at the wavelength of the test laser, but the figure of merit is low because of the high absorption. A better approach might be to move the peak absorption wavelength away from the laser wavelength (usually Nd-YAG light at 1.064 μm) until scattering of the light by nonuniformities in the film becomes the dominant loss mechanism. Winter *et al.*[1] suggest a compromise of putting the absorption peak at slightly shorter wavelengths than the laser and have tested two nickel dithiolate compounds absorbing at around 750 nm. The combination of a big nonlinearity and low absorption gives a figure of merit, *W*, of about 5. This figure, *W*, is the maximum refractive index change, at light intensities high enough to start damaging the material, divided by the absorption coefficient and the wavelength. It should be at least 3 and ideally more than 12 for good switching.

The materials most studied to date are the polydiacetylenes. Measurements on the *p*-toluenesulphonate substituted diacetylene produced a *W* of 0.15, away from the absorption[3]. Polydiacetylene films can be prepared by direct polymerization of a film of monomer but this results in high levels of scattering. Changing to a soluble polydiacetylene allows the film to be spin-coated onto a surface from solution, with much more uniform films and less scattering. Poly-4BCMU (another diacetylene derivative) can be put down in this way with a *W* that has been quoted[4] as 1.5. Unfortunately there is a catch. As the scattering is reduced two-photon absorption becomes a problem. This arises because the very high laser intensities allow absorption corresponding to twice the photon energy of the laser (532 nm wavelength). As this is in an absorption band of the red polydiacetylene, two-photon absorption is quite strong. Original estimates suggested that *W* could reach as high as 100, but this now looks unlikely.

The nickel dithiolates studied by Winter *et al.*[1] show low absorption at 532 nm and two-photon absorption does not seem to be significant. A further improvement in their efficiency might come from tuning the structure to shift the absorption peak up or down to optimize the balance of absorption and nonlinearity. It will also be necessary to build them into a polymer structure so that good clear films can be readily cast from solution. Scattering is prohibitively high for the films of crystals that can be made now. This also means that solution measurements on dithiolates are not really a fair comparison with actual waveguides made of diacetylenes because real waveguides are likely to have much more scattering, especially with early experimental samples.

Given all this it is not obvious that a *W* of 5 from a solution is going to be better than the diacetylene value of 1.5 measured on a real film. It is also likely that other undesirable effects, including degradation, will manifest themselves as we get closer to actual devices. It is clear that we are currently tuning molecular structures to optimize a fairly simple combination of spectral properties and we ought to have more predictive ability than we seem to have. What have the theoretical chemists been doing all these years? □

Paul Calvert is at the Arizona Materials Laboratories, University of Arizona, Tucson, Arizona 85712, USA.

---

1. Winter, C. S., Hill, C. A. S. & Underhill, A. E. *Appl. Phys. Lett.* **58**, 107–109 (1991).
2. Calvert, P. *Nature* **337**, 408 (1989).
3. Stegeman, G. I. Zanoni, R. & Seaton, C. T. *Mat. Res. Soc. Symp.* **109**, 53–64 (1988).
4. Townsend, P. D., Jackel, J. L., Baker, G. L., Shelburne, J. A. & Etemad, S. *App. Phys. Lett.* **55**, 1829–1831 (1989).

DAEDALUS

Food on the hop

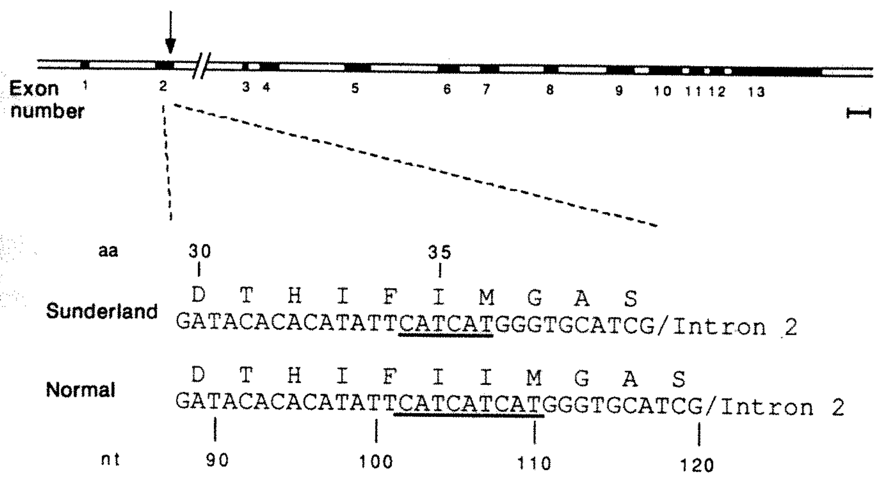
Now that biodegradable plastics are at last becoming available, Daedalus is widening the search. He is trying to produce a good biodegradable rubber. DREADCO's chemists point out that like rubber, many proteins exist in a folded-chain configuration, and some are highly elastic — like water-plasticized gelatin (table jelly) and elastin (the energy-storing component of the leg tendons of jumping animals like fleas and Kangaroos). The shape taken up by a given protein molecule is hard to predict, so rational design and synthesis of a elastic protein would be forbiddingly difficult. Instead, the DREADCO team are partially hydrolysing elastin, gelatin and other promising natural proteins, and recombining the fragments into novel 'artificial' (rather than fully synthetic) materials. Already they have produced a protein rubber so biodegradable that it is actually edible.

It seems a pity to waste such an unusual product on tyres, insulation and erasers, so DREADCO's marketeers plan to launch it as a foodstuff. They are reluctant to label it as a mere imitation meat. 'Substitute' or 'ersatz' products seldom acquire much dignity or sales; besides, the broiler industry already has a commanding lead in the production of rubber chicken. So the hunt is on for some uniquely valuable application for elastic food. Suggestions made to date include: pre-stressed 'novelty nibbles' that snap and ricochet beguilingly around inside the mouth when bitten; an all-edible elastic-powered clockwork mouse to give domestic cats the thrill of the chase without its cruelty; edible balloons for children's parties and edible rainwear for fetishists; boil-in-the-bag products that let you eat the bag as well; and (inspired by the pop-up toaster) a pre-stressed steak and a jumping sausage with meltable internal restraints, which leap dramatically out of the frying pan when they have reached the desired cooking temperature. But the main customers for specialized, outlandish, expensive foods are, of course, slimmers.

A number of slimming foods are designed to absorb water in the stomach, thus swelling up and giving the impression of fullness. Daedalus is devising an edible foam rubber which looks a bit like brown rice, but with grains that swell immensely once they have been swallowed. The slowly digestible foam core of each piece is compressed within a rapidly digested protein-rubber coating. In the stomach, the coating digests and ruptures, the foam expands wildly, and the consumer feels misleadingly full. Until the expanded pieces have been digested, they cannot even proceed into the narrow entrance to the small intestine. As long as they fill and block his stomach, the consumer continues to feel completely bloated. It may be hours before he can face another helping. David Jones

Deficiency in red blood cells

Sir — Glucose 6-phosphate dehydrogenase (G6PD) is a ubiquitous enzyme encoded by a gene that is highly polymorphic in humans[1]. Its deficiency is common in populations that have been subjected to selection by *Plasmodium falciparum* malaria[2]. Common variants entail the risk of acute haemolytic anaemia, but clinical expression of these variants is generally mild. By contrast, some individuals have rare variants associated with much more severe haemolysis[3]. The gene encoding G6PD has been mapped on the X-chromosome in mammals, specifically on Xq28 in humans[4].



Genomic structure of the human G6PD gene (top). The break symbolizes the large intron II. The arrow indicates the position of the mutation in G6PD Sunderland. Scale bar, 200 base pairs. Below, Amino acid and nucleotide sequence of a portion of exon II of G6PD Sunderland and of normal human G6PD. Amino acid (aa) numbers are on top, and nucleotide (nt) numbers are at the bottom. Numbering is from the beginning of the translated sequence (see in ref. 7). The CAT repeats, one of which is deleted in G6PD Sunderland, are underlined.

Kanno *et al.* claimed[5] that G6PD in red cells consisted of an amino-terminal region encoded by a gene on chromosome 6, and of a carboxy-terminal region encoded by the X-linked gene. In a News and Views article written at the time[6], L.L. commented that, although several arguments could be made against the likelihood of such a fusion protein, formal genetic evidence for X-linkage of the amino-terminal region of G6PD was not available, because none of the mutations reported at the time[7] mapped to that region. The claim by Kanno *et al.* has since been refuted by molecular analysis[8,9] and attributed to a purification artefact[10]. Nevertheless, mutations in the amino-terminal region have still failed to turn up[11].

Using a polymerase-chain-reaction based technique[12], we have now determined the nucleotide sequence of the entire coding region of the G6PD gene from a person with severe red cell G6PD deficiency and chronic haemolytic anaemia. The only abnormality we found was a three base-pair deletion in exon II (see figure), which predicts the loss of one of two adjacent isoleucine residues (amino acid 35 or 36), just upstream of the methionine residue called 'junctional' by Kanno *et al.* This part of exon II would not be expressed in red cells if the claim of Kanno *et al.* had been correct. Our observation demonstrates that a mutation in this X-linked amino-terminal region of G6PD causes deficiency in red cells.

This new type of G6PD, which we designate G6PD Sunderland, is the first variant found to result from a deletion rather than a base change. The deletion is within a threefold CAT repeat, and has presumably arisen through misalignment at meiosis, with conservation of the reading frame.

Donald MacDonald
Margaret Town
Philip Mason
Tom Vulliamy
Lucio Luzzatto

*Department of Haematology,
Royal Postgraduate Medical
School,
Hammersmith Hospital,
London W12 0NN, UK*

D.K. Goff

*Royal Infirmary,
Sunderland SR2 7JE, UK*

1. Luzzatto, L. & Battistuzzi, G. *Adv. hum. Genet.* **14**, 217–329 (1985).
2. Luzzatto, L. *Blood* **54**, 961–976 (1979).
3. Luzzatto, L. & Mehta, A. in *The Metabolic Basis of Inherited Disease* (eds Scriver, C. R., Beaudet, A. L., Sly, W. S. & Valle, D.) 2237–2265 (McGraw-Hill, New York, 1989).
4. Pai, G. S., Sprenkle, J. A., Do, T. T., Mareni, C. E. & Migeon, B. R. *Proc. natn. Acad. Sci. U.S.A.* **77**, 2810–2813 (1980).
5. Kanno, H., Huang, I.-Y., Kan, Y. W. & Yoshida, A. *Cell* **58**, 595–606 (1989).
6. Luzzatto, L. *Nature* **341**, 286–287 (1989).
7. Vulliamy, T. J. *et al. Proc. natn. Acad. Sci. U.S.A.* **85**, 5171–5175 (1988).
8. Mason, P. J., Bautista, J., Vulliamy, T. J., Turner, N. & Luzzatto, L. *Cell* **62**, 9–10 (1990).
9. Beutler, E., Gelbart, T. & Kuhl, W. *Cell* **62**, 7–8 (1990).
10. Yoshida, A. & Kan, Y. W. *Cell* **62**, 11–12 (1990).
11. Beutler, E. *Semin. Hemat.* **27**, 137–164 (1990).
12. Poggi, V., Town, M., Foulkes, N. S. & Luzzatto, L. *Biochem. J.* **271**, 157–160 (1990).

Sea-level constraints

Sir — The authors of three important papers[1–3] used Barbados coral cores of latest Pleistocene and Holocene ages to provide important constraints on sea-level fluctuations and to improve the radiocarbon timescale in this interval by calibrating it against the uranium–thorium timescale. The results of Bard *et al.*[3], for example, indicate that the last deglaciation started about 3,000 years earlier when referenced to the U–Th timescale than when referenced to the radiocarbon timescale. These authors suggest that this difference may explain the previously noted discrepancies between observations of sea-level change and models of glacial rebound in which ice sheets are considered only over Laurentia and Fennoscandia and that it is not necessary to introduce meltwater into the oceans from other ice sheets[4–6].

This conclusion is incorrect. Time enters into the formulation in three ways: through the history of the changes in the ice sheets; through the observations of sea-level change and through the Earth's rheology. Provided the timescales are linear relative to each other and provided that the same timescale is used throughout, the use of either the U–Th or the ^{14}C timescale is immaterial. The results of Fairbanks[1] indicate that the relation between the ^{14}C and U–Th timescales is effectively linear for the past 20,000 years and that any departures from this linearity are small when compared with the uncertainties associated with many of the radiocarbon ages used to constrain ice models and sea-level change.

The ice models are almost wholly constrained by radiocarbon ages of debris left by the retreating ice front. In some instances in Fennoscandia, varve chronologies have been used; these should be reduced to the radiocarbon timescale. (This does not appear to have been done but there are only few varve dates so they are unlikely to distort the global ice-sheet models.) Observations of sea level have sometimes been related to varve or U–Th timescales, but the bulk of reported data refers to the conventional radiocarbon timescale. In the rebound modelling, the Earth's viscous time constraint(s) are usually considered as unknowns so that the viscosities will be either in Pa (^{14}C)s or Pa (U–Th)s, depending on which timescale is used for the other two inputs. (The distinction is not so insignificant if the two timescales differ by about 3,000 years after 20,000 years.)

If the U–Th timescale for the Barbados corals is used to constrain the glacial-hydroisostatic rebound models then the ice models must be transformed to this timescale as well, with the result that the relation between observation and model prediction will be unchanged unless the relation between the two timescales is significantly nonlinear. One cannot usefully compare the U–Th timescale-controlled sea-level data with rebound predictions based on ice models linked to the ^{14}C scale. Yet this is what Bard *et al.* do.

A second difficulty with the conclusion of Bard *et al.* is the sea level during the last glacial maximum. Around 18,000–20,000 years ago, sea levels are generally believed to have been at about 130 m below their present level (although this depth will be regionally variable) and the Barbados coral data support this: the minimum depths at this time of the corals are about 120 m below present sea level. Ice models for the Laurentide and Fennoscandia that produce rebound at the centres of these ice sheets that are consistent with these observations of sea-level change within and near the ice-sheet margins contain only enough ice to raise sea level by 60–80 m (refs 5,7). Increasing the volumes in these ice sheets results only in implausible predictions of rebound at these sites unless the melting was initially very rapid. The Barbados sea-level curve from 18,000 to 6,000 years before present, however, argues against this, so it has been suggested that this missing melt water has to come from other areas such as the Barents–Kara ice sheet in Antarctica, or possibly from eastern Siberia[4–6]. Rather than negate the previously drawn conclusions about the importance of these centres of deglaciation, the Barbados results reinforce them.

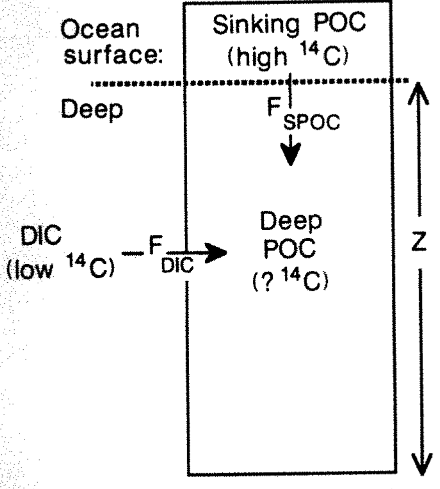
Kurt Lambeck
Research School of Earth Sciences,
Australian National University,
Canberra, ACT 2601,
Australia

Masao Nakada
Department of Geology,
Faculty of Science,
Kumamoto University,
Kumamoto 860, Japan

1. Fairbanks, R.G. *Nature* **342**, 637–642 (1989).
2. Bard, E., Hamelin, B., Fairbanks, R. G. & Zinder, A. *Nature* **345**, 405–410 (1990).
3. Bard, E., Hamelin, B. & Fairbanks, R. G. *Nature* **346**, 456–458 (1990).
4. Nakiboglu, S. M., Lambeck, K. & Aharon, P. *Tectonophysics* **91**, 335–358 (1983).
5. Nakada, M. & Lambeck, K. *Nature* **333**, 36–40 (1988).
6. Peltier, W. R. *Quat. Res.* **29**, 93–112 (1988).
7. Peltier, W. R. & Andrews, J. T. *Geophys. J. R. astr. Soc.* **46**, 605–646 (1976).

Another recipe for bomb ^{14}C dilution

Sir — As recently pointed out by Druffel and Williams[1] and Toggweiler[2], the relatively low amount of atom-bomb-derived ^{14}C in deep-ocean particulate organic carbon (POC) has significant implications for the way carbon is cycled in the ocean's interior. Druffel and Williams conclude that the low $\Delta^{14}C$ found in POC suspended in the deep ocean is probably the result of dilution by incorporation of ^{14}C-depleted dissolved organic carbon (DOC) into deep POC. As well as DOC→POC transformations, I wish to point out that dissolved inorganic carbon (DIC) is another large carbon source for such dilution, and that the conversion rate of DIC to POC in the deep sea is quantitatively, and therefore isotopically, important.



Model of deep-ocean POC formation from dissolved inorganic carbon (DIC) and sinking POC sources. Within the deep ocean, sinking and suspended POC are not differentiated. F_{spoc}, areal flux rate of sinking POC through the surface/deep ocean boundary; F_{dic}, areal flux rate (uptake) of deep DIC into POC.

Let us assume that the only source of non-bomb carbon in the deep ocean is DIC, and that deep POC must therefore originate from some combination of this source and ^{14}C-rich sinking POC (see figure). If we know the fluxes and $\Delta^{14}C$ of these sources, the $\Delta^{14}C$ in POC resident in the deep ocean can be calculated. Although it has been assumed[1] that uptake of DIC into POC is negligible in the deep sea, Sorokin reported[3] uptake rates ranging from 0.5 to 3 μg C m^{-3} d^{-1} at depths greater than 1 km in the central Pacific Ocean. Such rates are indeed minuscule when compared to those in surface waters, but they become significant when integrated over depth and compared with the sinking POC flux into the deep ocean. Using a mean deep-ocean DIC uptake rate of 2 μg C m^{-3} d^{-1}, the estimated areal flux rate of DIC into POC (10 mg C m^{-2} d^{-1}) equals the sinking POC flux into deep water in the north central Pacific Ocean (see table). This yields an estimated deep POC $\Delta^{14}C$ of −52 ‰ (see table), even more depleted in bomb ^{14}C than actually observed[1] (> 43 ‰), indicating the potential importance of the DIC → POC pathway in effecting ^{14}C dilution in the deep sea.

In the Santa Monica Basin, a greater flux (30 mg C m^{-2} d^{-1}) of POC sinking into a shallower aphotic zone could be viewed as less advantageous for dilution of bomb ^{14}C in deep POC. But aphotic DIC→POC conversion in such basin environments ranges from <1 to 24 mg C m^{-3} d^{-1} (refs 4–7), at least several orders of magnitude greater than those observed[3] in the open ocean. Assuming a conservative mean rate estimate for this site of 1 mg C m^{-3} d^{-1}, depth-integrated uptake of deep DIC is calculated to be 800 mg C m^{-2} d^{-1}, or more than 20 times the flux entering aphotic waters via sinking POC. This again yields an estimated deep POC $\Delta^{14}C$ value that is much lower than that of POC sinking from surface waters (see table).

A further example of the magnitude of such a process is provided by Tuttle and Jannasch[5], who reported that DIC uptake in waters above the Cariaco trench is at least 17–58 % as great as photosynthetic inorganic carbon assimilation in overlying surface waters. The above observations again lead to the conclusion that bomb ^{14}C in resident deep-ocean POC can be substantially diluted by DIC uptake, independent of DOC incorporation or other mechanisms[1].

FLUXES AND $\Delta^{14}C$ RELEVANT TO DEEP POC FORMATION

		Fluxes		$\Delta^{14}C$		
Site	*Z*	SPOC	DIC	SPOC	DIC	DPOC
	(m)	(mg C m^{-2} d^{-1})		(‰)		
NCP	4,760	10	10	+136	−240	−52
SMB	800	30	800	+86	−50	−45
Ref.	1	8,9	3–7	1	1	(This report)

SPOC, sinking POC at surface/deep ocean boundary; DPOC, deep POC (both sinking and suspended); NCP, north central Pacific Ocean site[1]; SMB, Santa Monica Basin site[1]. The DIC flux (F_{dic}) is derived by multiplying the mean per-volume rate estimates (2 μg C m^{-3} d^{-1} (ref. 3) and 1 mg C m^{-3} d^{-1} (refs 4–7) for NCP and SMB, respectively) times the respective deep-ocean water column depth, *Z*. The surface/deep ocean boundary is arbitrarily chosen to be at a depth of 1,000 m at the NCP site and at 100 m at the SMB site. DPOC $\Delta^{14}C = ((F_{\mathrm{spoc}}/F_t) \times \mathrm{SPOC}\ \Delta^{14}C) + ((F_{\mathrm{dic}}/F_t) \times \mathrm{DIC}\ \Delta^{14}C)$ where $F_t = F_{\mathrm{spoc}} + F_{\mathrm{dic}}$. Δ^{14} (ref. 1) in units of parts per thousand (‰).

Thus, as previously noted[10], DIC uptake should not be ignored in interpreting the paucity of ^{14}C in some deep-sea organisms and POC pools. It would therefore be premature to ascribe such ^{14}C depletion to DOC incorporation alone. Clearly, further rate and isotope measurements are needed better to characterize this important piece of the global carbon cycle.

Greg H. Rau
Institute of Marine Sciences,
University of California,
Santa Cruz, California 95064 USA

1. Druffel, E.R.M., & Williams, P.M. *Nature* **347**, 172 (1990).
2. Toggweiler, J. R. *Nature* **347**, 122 (1990).
3. Sorokin, Yu. I. *Oceanology* **11**, 85 (1971).
4. Seki, H. & Zobell, C. E. *J. Oceanogr. Soc., Japan* **23**, 182 (1967).
5. Tuttle, J. H. & Jannasch, H. W. *Limnol. Oceanogr.* **24**, 746 (1979).
6. Seki, H. *et al. Arch. Hydrobiol.* **100**, 73 (1984).
7. Juniper, S. K. & Brinkhurst, R. O. *Mar. Ecol. Prog. Ser.* **33**, 41 (1986).
8. Martin, J. H. *et al. Deep-Sea Res.* **34**, 267 (1987).
9. Nelson, J. R. *et al. Cont. Shelf Res.* **7**, 307 (1987).
10. Rau, G. H., Karl, D. M. & Carney, R. S. *Deep-Sea Res.* **33**, 349 (1986).

Scientific Correspondence

Scientific Correspondence is intended to provide a forum in which readers may raise points of a scientific character. They need not arise out of anything published in *Nature*. In any case, priority will be given to letters of fewer than 500 words and five references. □

cDNA SYNTHESIS

Like Great Research, MagneSphere™ Technology Advances One Step At A Time

Capture Clone™ cDNA synthesis system... The Next Logical Step

Promega introduces Capture Clone cDNA synthesis and ligation system utilizing Promega's MagneSphere magnetic particle technology.

- **Easy - no spin columns, phenol/chloroform extractions or precipitations**
- **Versatile - includes *Xba* I/*Not* I polylinker primer and *Eco*R I adaptor**
- **Complete - includes all components necessary for vector-ready cDNA**
- **Fast - one day protocol**

...from Promega

PolyATtract™

mRNA isolation system utilizing MagneSphere magnetic particle technology.

- **Fast - total RNA to message in 45 minutes**
- **Easy - no oligo (dT) columns or centrifugation**

For complete information on Promega's MagneSphere technology products, call 800-356-9526.

Promega

Worldwide Contact:		Denmark	42 94 88 22	Israel	3 430 876	Norway	353 8488	South Africa	11 886 4710
Australia	2 810 8480	Finland	0 708 081	Italy	55 67 64 41	P.R. of China	1 201 3616	U.K.	0800 378 994
Austria	222 39 35 27	France	1 45 33 67 17	Japan	3 270 0966	Singapore, Malaysia, Thailand		Taiwan	2 3923078
Belgium, The Netherlands,		Greece	01 644 0547	Korea	2 588 5961	and Indonesia	339 1691	Venezuela and	
Luxembourg	71 21 71 21	India	11 684 6565	Mexico	5 543 3630	Spain, Portugal	93 404 52 20	Colombia U.S.#	201 467 6505
Canada	800 267 7424	Ireland	1 426644	New Zealand	9 521 3100	Sweden, Iceland	346 83050	West Germany	06221 502144

Promega Corporation
2800 Woods Hollow Road
Madison, WI 53711-5399 USA
Toll Free 800-356-9526
Telephone 608-274-4330
FAX 608-273-6967
Telex 62057092

Reader Service No.443

800-356-9526

Update your Springer Library

Recent Titles in Earth Sciences

N. D. Chatterjee

Applied Mineralogical Thermodynamics

Selected Topics

1991. XVI, 321 pp. 79 figs. 66 tabs. Softcover DM 98,-
ISBN 3-540-53215-3

Contents: 1. Summary of basic thermodynamic concepts: a refresher. - 2. Measurement, evaluation, and tabulation of thermodynamic properties. - 3. Equations of state for fluids and fluid mixtures. - 4. Phase relations among end-member solids. - 5. Phase relations among end-member solids and a pure fluid. - 6. Phase relations among end-member solids and a binary fluid mixture. - 7. Derivation of an internally consistent thermodynamic dataset by mathematical programming. - 8. Thermodynamics of crystalline solutions. - 9. Phase equilibria involving nonideal solutions and outlines of geothermometry and geobarometry. - References. - Subject Index.

E. G. Kauffman, O. H. Walliser (Eds.)

Extinction Events in Earth History

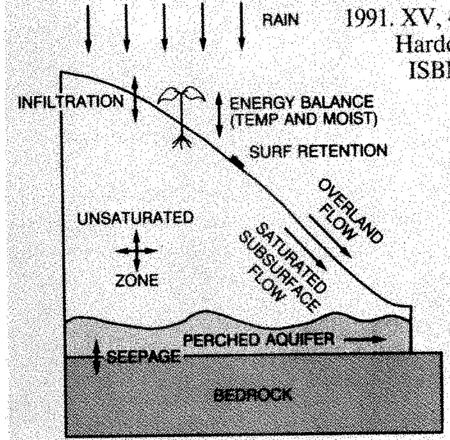
Proceedings of the Project 216: Global Biological Events in Earth History

1990. VI, 432 pp. (Lecture Notes in Earth Sciences, Vol. 30) Softcover DM 75,- ISBN 3-540-52605-6

T. J. Schmugge, J-C. André (Eds.)

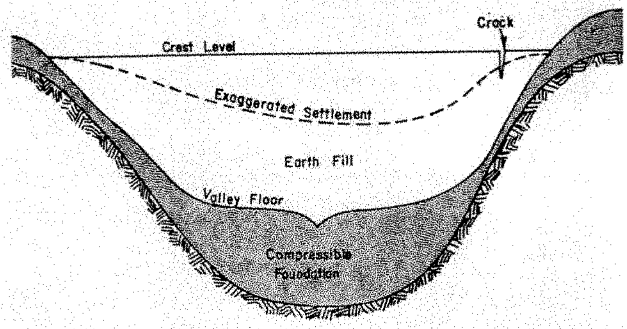
Land Surface Evaporation

Measurement and Parameterization



1991. XV, 424 pp. 249 figs.
Hardcover DM 168,-
ISBN 3-540-97359-1

For further information, please contact your bookseller, use the Nature Reader Service Card, or write directly to Springer-Verlag. Prices are subject to change without notice.



R. H. Bennett, W. R. Bryant, M. H. Hulbert (Eds.)

Microstructure of Fine-Grained Sediments

From Mud to Shale

W. A. Chiou, R. W. Faas, J. Kasprowicz, H. Li, T. Lomenick, N. R. O'Brien, S. Pamukcu, P. Smart, C. E. Weaver, T. Yamamoto (Associate Eds.)

1991. XXII, 582 pp. 458 figs. in 708 parts (Frontiers in Sedimentary Geology) Hardcover DM 198,-
ISBN 3-540-97339-7

R. Riding (Ed.)

Calcareous Algae and Stromatolites

1991. XI, 571 pp. 202 figs. 23 tabs. 16 plates.
Hardcover DM 228,- ISBN 3-540-52373-1

P. Brosche, J. Sündermann (Eds.)

Earth's Rotation from Eons to Days

Proceedings of a Workshop Held at the Centre for Interdisciplinary Research (ZiF) of the University of Bielefeld, FRG, September 26-30, 1988

1990. XV, 255 pp. 89 figs. 25 tabs. Hardcover DM 128,-
ISBN 3-540-52409-6

Earth's Rotation from Eons to Days reviews long-term changes, methods of measurement, and the major influences on rotation parameters. In order to understand secular changes, the momentary behavior of ocean tides must be analyzed and appropriately modelled. Researchers and students in astronomy and all fields of geosciences will find a wealth of information related to the interaction of geophysical phenomena and the rotation of the planet Earth.

Springer-Verlag
Berlin
Heidelberg
New York
London
Paris
Tokyo
Hong Kong
Barcelona

☐ Heidelberger Platz 3, W-1000 Berlin 33, F.R. Germany
☐ 175 Fifth Ave., New York, NY 10010, USA
☐ 8 Alexandra Rd., London SW19 7JZ, England
☐ 26, rue des Carmes, F-75005 Paris, France ☐ 37-3, Hongo 3-chome, Bunkyo-ku, Tokyo 113, Japan
☐ Room 701, Mirror Tower, 61 Mody Road, Tsimshatsui, Kowloon, Hong Kong ☐ Avinguda Diagonal, 468-4° C, E-08006 Barcelona, Spain

tm.30.076/SF

Disease of war

Alastair Hay

Preventing a Biological Arms Race. By Susan Wright. *MIT: 1990. Pp.446. £22.50, $25.*

IF an image were required to sum up what biological warfare is about, it could be the remnants of the bombed baby-milk factory on the outskirts of Baghdad. The United States claims that it was a biological weapons plant, but Iraq says it was nothing other than a factory formulating powdered milk into infant feeds. Who do you believe?

A journalist on the *Washington Post* diligently tracked down the plant manufacturer (in New Zealand) which maintains that it was indeed built to process powdered milk. Apparently the factory was closed for a time and then restarted by French engineers, again to handle powdered milk. But, says the United States, it was after August 1990, and after the French had left, that the factory was used for nefarious purposes. And why, the Americans ask, was it in camouflage livery? Iraq says the camouflage can be explained simply by the availability of building material when the plant was constructed.

There is a sense of unreality about this. In fact much of the ruminating about biological warfare is unreal. So much information is shrouded in a veil of secrecy. At a time like this, anything that serves as a reminder that it is not just make-believe, or not all make-believe, is welcome. *Preventing a Biological Arms Race* is just such a reminder. Edited by Susan Wright, the book is a systematic collection of essays written by participants in the biological warfare debate. Few stones are left unturned in chapters that range over the origins of the US Biological Warfare Program (the United States destroyed its biological weapons between 1970 and 1973), the potential of genetic engineering to modify existing diseases for use in munitions, the role of international law and, of course, allegations about biological warfare.

Fortunately, for the moment, we are still only talking about allegations. But when the United States accuses the Soviet Union of violating the two international treaties that govern conduct in this area, the 1925 General Protocol and the 1972 Biological Weapons Convention, both of which outlaw biological warfare, the allegations must be taken seriously.

According to the United States, the first foul occurred in the Soviet city of Sverdlovsk in 1979. The problem was anthrax, caused, says the United States, by the release of airborne spores following an explosion at a biological weapons facility. Release of the spores was said to have caused pneumonia in some people and, therefore, the most likely candidate was pulmonary anthrax, an agent considered by the United States at one time as a suitable biological weapon. The Soviet response acknowledged that an outbreak of anthrax had occurred, but claimed that it was gastric in origin, and caused by the consumption of contaminated meat. Anthrax is still endemic in parts of the Soviet Union.

Support for the more prosaic Soviet version of events is provided in an interview given to the *New York Times* in 1981 by Donald Ellis, then professor of physics and chemistry at Chicago's North Western University. Ellis was in Sverdlovsk at the time of the alleged incident, and said that he regularly passed unhindered within a few blocks of the site on his way to and from work. Ellis felt that if there had been a release of anthrax spores, the Soviet authorities would have taken steps to protect him. Rumour has it that Ellis was not the only visiting foreign scientist; half a dozen others were also in the city at the time and were apparently allowed to move freely about its precincts.

Popperfoto

Biological weapons plant or baby-milk factory — who do we believe?

Regrettably, the Soviet authorities neither invited senior US scientists to visit the site of the accident nor did they offer them medical records to examine. Prudence might have suggested that an invitation would have been wise, as allegations have a tendency to stick unless refuted. Although there is no requirement under the existing treaties for countries to allow inspections of sites that are the subject of dispute, the Soviet reluctance to have outsiders take a look has only fueled speculation that it had something to hide.

In view of the seriousness of the Sverdlovsk incident, it would have been extremely valuable to have had a Soviet contributor to this book addressing some of the issues raised in its chapters. Had such a contributor been found, he/she would rightly have poured scorn on the second of the American allegations, that is that the Soviet Union had encouraged the use of fungal toxins as chemical/biological warfare agents in Southeast Asia between 1978 and 1983. These incidents, known in the trade as 'yellow rain' following the description — by a Hmong soldier from Laos — of an alleged spray attack, have come to resemble a farce. We now know, improbable though it seems, that what was thought to be a sophisticated delivery system for toxins using deproteinated pollen and uric acid, was nothing other than the excreta of Asian honey bees.

Yellow rain has now been thoroughly debunked. Scientists and others in many countries have assisted in the systematic dismantling of the US case. The most effective sceptic was Matthew Meselson, professor of biochemistry and molecular biology at Harvard. In a chapter that explains the allegations and the evidence for and against, Meselson and two colleagues point out how the Reagan administration locked itself into a "political position from which it has yet to extricate itself". The administration had only itself to blame. By failing to corroborate testimonies of witnesses, ignoring pleas by Department of Defense scientists for more time to confirm findings, and bypassing its own scientific advisers, the administration broke all bureaucratic rules by going public on the flimsiest evidence.

Internal rules partly govern the way governments behave, but international rules are also needed to keep them in check. Given its use of chemical weapons against Iranian soldiers and Kurdish civilians, Iraq clearly needs the constraints of international opinion. But from this distance it seems improbable that Iraq would house a biological weapons plant in a corrugated iron building. In view of the serious risk of local contamination it is even more questionable that Iraq would then allow journalists to trample over the bombed site to witness torn sacks of powdered milk. A far more likely site, if indeed there is one, would be the pharmaceutical plant said to be two miles down the road.

Whether or not Iraq has biological weapons remains a subject of speculation. Sadly, what is no longer just speculation is the growing body of evidence — amply documented in this book — that biological warfare is being viewed again by some sections of the military as something that may be of value. The counterweight to this is the evidence that most governments respect, and wish to strengthen, the Biological Weapons Convention. The chance to do this will occur later this year when the convention undergoes its third review (the last was five years ago). The contributors to *Preventing a Biological Arms Race* have helped to focus attention on the additional measures that are needed to support the convention, and to thwart the military interest in biological warfare. For thwarted it must be. ☐

Alastair Hay is in the Department of Chemical Pathology and Immunology, Old Medical School, Leeds LS2 9JI, UK.

Mostly about aluminium

Robert W. Cahn

R & D for Industry — A Century of Technical Innovation at Alcoa. By Margaret B. W. Graham and Bettye H. Pruitt. *Cambridge University Press: 1991. Pp.645. $49.50, £50.*

SOME years ago, the *New Yorker* carried one of its 'cocktail party' cartoons: a group of stolid businessmen is conversing, and one declares: "I've learnt a lot in 62 years, but unfortunately most of it is about aluminum." Drs Graham and Pruitt have written a massive book that on the face of it is also mostly about alumin(i)um — but it is also about much more. The authors (one of whom is professor of operations management at Boston University) are associated with the Winthrop Group, a company which specializes in the history of business and technology. The book, in the guise of an exhaustive history of the research laboratory of the Aluminum Company of America, is really about the permanent, crucial and insoluble problem of the proper relationship between commercial management and research manangement in a large industrial company. As the authors point out, aluminium as an industrial material, and corporate industrial research and development are both about a century old; their crises and triumphs developed in synchrony.

Although the German chemical industry already had large research laboratories in the 1880s, it is generally accepted that the first true corporate industrial R&D laboratory in America was set up in a Schenectady barn in 1900 by Willis R. Whitney for the General Electric Company (see the splendid biography *Willis R. Whitney, General Electric and the Origins of US Industrial Research* by George Wise, Columbia University Press, 1985); before that, there had only been troubleshooting laboratories attached to individual works. After 1900, the idea of segregating corporate researchers in a separate location, sheltered to some degree from day-to-day operating crises, rapidly gained ground. Du Pont already had its Experimental Station in 1902, and its history has been exhaustively analysed in another excellent book recently published by the Cambridge University Press (David A. Hounshell and John K. Smith, *Science and Corporate Strategy, Du Pont R&D, 1902–1980*, 1988). Most of the points made about that very different company find their echoes in the new book about Alcoa.

New in paperback

The Origins of our Universe by Malcolm S. Longair is based on the authors 1990-91 Royal Institution Christmas Lectures. The book is published by Cambridge University Press; price £7.95, $12.95 (also available in hardback; price £20, $34.50.

Fields, Strings and Quantum Gravity edited by Hanying Guo, Zhaoming Qiu and Henry Tye is published by Gordon and Breach and examines the present status of string theory, quantum gravity and conventional field theory in theoretical physics. Price is $105.

Chapman and Hall recently published *Monitoring for Conservation and Ecology* at a price of £14.95, $35. The editor, F. B. Goldsmith brings together a team of ecologists and conservationists to provide a useful summary of ecological monitoring.

Alcoa's business was built on the patent granted in 1889 to Charles Martin Hall, the co-inventor (with Frenchman Paul Héroult) of the electrolytic process still used today for smelting aluminium. Hall became a technical director of the fledgling company, a position which he used to run a small group of subordinates within a factory, under his close direction. He defended his personal authority with tigerish determination when he refused, in 1909, to permit a corporate research laboratory to be established, a determination which he maintained even when he was dying of leukaemia some years later. In consequence, the laboratory was established only in 1919, and the company suffered greatly from this late decision. The battle of Hall versus the rest of the board of directors was a precursor of many later battles about the size, ethos and degree of independence of the company's successive R&D laboratories, battles which swung first one way and then another, culminating in the construction of the Alcoa Technical Center, where research (development moved in earlier) began in 1972.

There is space here only to list some of the perennial issues discussed in the light of the company's history: project control (in R&D) by a system of standing committees set up by commercial management versus a role for long-range research under the control of a director of research; protection of company interests by patenting versus secrecy without patents; whether it was safe to accept Government money for research (for years it was thought unsafe, a direct consequence of the Justice Department's initiation of anti-trust action in 1937, an action which dragged on for years and obliged Alcoa to hand over a number of patents). Other issues included whether researchers could safely be encouraged to participate fully in the professional life of their various scientific specialities versus a policy of purdah, to protect company secrets; developing detailed processing technology for novel uses of the company's products versus leaving that to customers (this was a very major issue over the introduction of aluminium alloys for beverage containers); what resources it was proper to devote to the effort to replace the Hall-Héroult process by a radically different way of making aluminium (this expensive and sustained effort came to nothing, but during its time powerfully buttressed the reliance on secrecy with all its attendant problems).

The later part of the book recounts the decision in the 1980s to recast research strategy in a very radical way, partly as a result of five years' pressure by an outsider brought in to advise on R&D policy. Long-range research was instituted to give the company completely new options to diversify, in due course, from aluminium, greatly increasing total R&D expenditure and also the number of patents taken out; the number of staff with doctorates was doubled, which of necessity led to increased involvement of staff in the activities of their professional bodies, and obliged Alcoa to institute an in-house system of awards to distinguished researchers. In spite of the success of the new R&D strategy, there was further boardroom anxiety about the cost, leading in 1987 to the replacement, by an outsider, of the company chairman who had masterminded the strategy. The creative tension between the commercial ethos and the research ethos, with right on both sides, can never be finally resolved; the current problems at the Philips Research Laboratories show just how difficult this tension is.

This is a profound and stimulating book, always analytical, never hagiographic, rarely censorious. It is a distinguished part of the new wave of industrial history, and well worth the attention of any applied scientist, irrespective of his speciality. ☐

Robert W. Cahn is in the Department of Materials Science and Metallurgy, Cambridge University, Pembroke Street, Cambridge CB2 3QZ, UK.

Multiple scanning probes

Graham Dunn

Confocal Microscopy. Edited by T. Wilson. *Academic Press: 1990. Pp.426. £33, $71.*

IN a charming memoir recounting his invention of the confocal scanning microscope in 1955, Marvin Minsky recollects that it was fortunate that his brother-in-law was a patent attorney, otherwise the instrument might never have been documented at all. It was fortunate indeed for biologists because, in combination with lasers, confocal scanning microscopy has now become indispensable for exploring the three-dimensional structure and dynamics of living systems. In the words of Shinga Inoué: "Seldom has the introduction of a new instrument generated as instant an excitement amongst biologists . . ." Even so, the full potential of confocal scanning microscopy is still far from realized and this latest book on the subject contains a fascinating insight into possible future developments of this very versatile technique.

Confocal Microscopy starts with a heavy chunk of theory — indigestible for many, but invaluable to readers interested in developing a confocal microscope or getting the most out of an off-the-shelf instrument. The first and third chapters are mainly concerned with a comprehensive survey of the theory of confocal imaging by the master himself: Tony Wilson. This is supplemented in chapter 5 by a sophisticated treatment of spatial filtering at the confocal pupils. Stephen Hawking was warned, when preparing his popular book *A Brief History of Time* (Bantam, 1988), that every equation halves the readership. If true, this bodes very ill for the popularity of this book, which has 106 equations in Wilson's two chapters alone! Nevertheless, the nontechnical reader who persists with these chapters and takes the derivation of the equations on trust will be rewarded by a thorough knowledge of the principles of confocal microscopy.

Chapter 2, 4, 6 and 7 are concerned mainly with the theory and practice of three-dimensional image representation and optical sectioning in confocal microscopy. Although the first seven chapters describe many of the latest advances and are essential reading for any serious confocal microscopist, it is the second part of the book that, for me, contains a vision of the future. Chapters 8 and 15 discuss the latest developments in phase contrast and interference techniques for the confocal microscope and chapters 9 and 14 discuss the theory and application of Nipkow disc instruments. These confocal microscopes are often considered the poor cousins of stage scanning and mirror scanning instruments but their feature of multiple scanning probes may eventually prove a decisive advantage for high-speed, three-dimensional scanning of living tissue. Taken together, advances in these areas could lead to instruments capable of recording in great detail the full four dimensional space/time structure of the early living embryo using noninvasive optical contrasting techniques. Further speculation on the direction of future developments in cell biology is to be found in chapter 11, whereas the remaining chapters discuss the latest confocal techniques in other major areas of application, including semiconductor metrology and ophthalmology.

In considering the book's potential market, it is easier to say what the book is not rather than what it is. It is certainly not intended as a 'Which Confocal Microscope?' buyer's guide and it is unfortunate for those seeking such advice that Wilson considers it invidious to compare products. Nor is it a practical guide to using confocal microscopes: although much practical advice can be gleaned from it I would suggest (though I hope not invidiously) that biologists will probably find more in the recent *Handbook of Biological Confocal Microscopy* (Plenum, 1990) edited by James Pawley. Aristotle is reputed to have said that 'When a thing has been said once it is hard to say it differently', and it is true that these two books have much in common. Yet I think that Wilson's book, giving a more thorough and cohesive treatment of the principles of confocal microscopy, will appeal more to designers and developers, whereas users might favour Pawley's book. I am not sure whether either book qualifies as a *vade mecum* but I suspect that the true aficionado of confocal microscopy will not wander far without his or her copy of Wilson. ☐

Graham Dunn is in the MRC Muscle and Cell Motility Unit, Department of Biophysics, King's College London, 26 — 29 Drury Lane, London WC2B 5RL, UK.

Inscrutable matter

John L. Friedman

Black Holes and the Universe. By Igor Novikov. *Cambridge University Press: 1990. Pp.176. Hbk £25, $39.50; pbk £8.95, $14.95.*

By 1784, it was already clear to the English physicist John Michell that there might be stars large and dense enough that, to escape their surface, one must exceed the speed of light. "Since their light could not arrive at us . . . we could have no information from sight. Yet, if any other luminous bodies should happen to revolve around them, we might still perhaps from the motions of these revolving bodies infer the existence of the central ones with some degree of probability". Objects from the familiar world of newtonian physics, Michell's black stars are curious but easily understood. More difficult to grasp is their modern incarnation, the black holes of general relativity. Novikov has written, for the scientifically literate layman, an entertaining, and unusually detailed introduction to these realistic black holes and the universe that they apparently pervade.

If gravity were really newtonian, the stellar corpses that Novikov describes would be denser than other stars and dimmer, but neither black nor holes. Light could travel outward from their surface before falling back, and it would be possible to observe them. In fact, with a sufficiently powerful spacecraft, one could visit them and return. But in the real universe, the gravity of a star dense enough to trap its own light has strength to bend inward the path of all its matter. Nucleons, electrons, neutrinos and light fall to a centre, their extent collapsing to the circumference of a speck. Inside the speck, density and curvature are too high for present physics to describe. Outside the speck space is empty, remembering in its mathematically precise curvature only the mass and rotation of a vanished star. Because the space between speck and horizon is empty, and because any matter crossing the horizon falls inward, 'black hole' is a plausible metaphor. Novikov quotes the poet Marina Katys: "a gulf against the shores of space and time".

Novikov is the latest in a sequence of experts in the field to portray without mathematics the remarkable role that general relativity has come to play in astonomy. The famous co-author (with Y.B. Zel'dovich) of an indispensable text in relativistic astrophysics has given us a more complete picture of black-hole astrophysics than one finds in the similar short books by Robert Wald (*Space, Time, and Gravity: The Theory of the Big Bang and Black Holes,* University of Chicago Press, 1977) and Stephen Hawking (*A Brief History of Time: From the Big Bang to Black Holes,* Bantam, 1988). His discussion of black holes may be less accessible than Hawking's, but Novikov's own pioneering contributions (including the first published prediction with Zel'dovich and Doroshkevich of the 3K cosmic microwave background radiation) more often involve the big bang than black holes. With Marina Katys' poetry, a Soviet song, and his own anecdotes, Novikov leads us to his truer love. He has a talent for telling stories of scientific discovery, and his portrayal of the young science of physical cosmology is a drama of unexpected brilliance and blindness. ☐

John Michell — contemplating the existence of black stars.

Mary Evans

John L. Friedman is in the Department of Physics, University of Wisconsin, Milwaukee, Wisconsin 5320, USA.

Growing pains

Stuart Sutherland

Human Change Processes: The Scientific Foundations of Psychotherapy. By Michael J. Mahoney. *Basic Books: 1991. Pp.590. $45.*

SCIENCE has two main aspects, the systematic recording of observations and the construction of rigorous theories to explain them. The subtitle of *Human Change Processes: The Scientific Foundations of Psychotherapy* should constitute an offence under the Trades Descriptions Act: there is nothing in the whole book that remotely resembles either the data or the theories of science. It is the sort of book that brings psychology into disrepute, which is a pity as even on the difficult problem of psychotherapy one or two facts have been established, even if there is still nothing meriting the name of theory.

The book starts with a potted history of philosophy, ranging from Heraclitus (who you will remember never stepped into the same river twice) to Kant. Next, it purports to give a history of 'Scientific Studies of Human Learning and Development'. Although the word 'scientific' keeps popping up in unexpected places, we are treated only to a stream of opinions, many of them banal, such as "David Foulkes studied the dreams of 150 children . . . That research [perish the word] has portrayed dreams as . . . predominantly based in (or drawing upon) what we know and remember". Here is another of Michael Mahoney's insightful conclusions: "To sum up, there is no question that memory and what we experience as mental representations are important phenomena in our lives." He fares no better with the 'theories' he reviews, an activity he describes as 'metatheorising'. "Constructionism has been used with two different meanings: as a portrayal of the organism as an active agent in its own ongoing development, and as a means of highlighting the social contexts that construct and orient our efforts at knowing, communicating, and becoming." When translated into English, this would appear to mean that social scientists believe that although we are influenced by others, we take our own decisions.

Mahoney provides little in the way of argument. He supports his idea that "self examination is important in optimal psychotherapy" by presenting the results of a poll he conducted among psychotherapists: 2,000 psychotherapists cannot be wrong. Or again, he conveys the depressing news that "there are now dozens of distinguishable theories of emotionality, hundreds of volumes devoted to that topic, and tens of thousands of articles dealing with various aspects of human affect." He does not stoop to tell his readers what has been found out about the emotions, perhaps wisely for almost nothing worth knowing has been discovered.

The American bison — about 50,000 remain from an original population of 60 million. Taken from *The Natural History of North America* by Edward Ricciuti (Facts on File, $35, £18.95).

Having listed the opinions on human development of everyone he can think of and having given them all a prize for their sagacity, if not for their ability to prove their point, he turns in the final section, which has nothing to do with the other two, to the techniques of psychotherapy. Apparently it helps his patients if they look at themselves in a mirror, utter a series of random thoughts, dance, meditate, have massage, play different roles, indulge in 'private dialogue' and write an autobiography (one hopes not for publication). He notes that his patients 'oscillate': remarkably, they are happier at some times than at others. Mahoney never considers such questions as whether psychotherapy is a useless self-indulgence, except possibly for the mentally disordered. The majority of its clientele in America appear to be mentally normal: they just want to 'grow'. Despite Mahoney's assertion to the contrary, it is rare to see anyone over 30 or 40 growing, though they often change for the worse. Nor does he question the desirability of examining the patient's past, which cannot be changed, rather than persuading him to examine his future over which he has some control. Mahoney complains that "Federal funding for research in the social and behavioural sciences has been reduced by 25%". Judging by this book, the American Government has at last got something right.

He has a naive faith in his own success as a therapist. There are now many studies of the outcome of therapy: the sort he espouses, namely dynamic therapy, does no better than a placebo control group in which a sham therapist simply allows the patient to talk and occasionally makes supportive noises. Moreover, despite Mahoney's insistence on the need for training, it has been shown that completely untrained counsellors do precisely as much or as little good as those who have been trained. He is unable to understand that if you have made sacrifices to enter a profession and if you are now making a decent living out of it, you are likely to think that you are doing some good even if you are not.

Despite his ability to cover nearly 600 pages with verbiage, Mahoney is clearly a very nice man. He actually submits to psychotherapy himself because he feels that only by 'growing' and learning how to 'cope' with his problems can he be of maximum use to his patients. There is fortitude and self-sacrifice indeed.

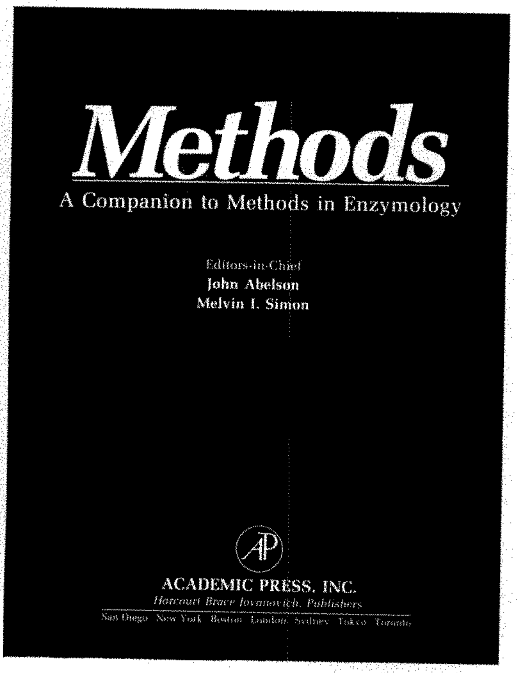
Occasionally Mahoney says something that needs saying, perhaps after the fashion of the celebrated monkeys who wrote the whole of Shakespeare by scribbling at random. For example, he rightly attacks those psychotherapists who preach to their patients that it is all in their heads on the grounds that "The parameters responsible for the pain of starvation, rape, and oppression are not confined to the nervous system of their victims." Moreover, some of his case histories are interesting. The patient always emerges a better person, but, be warned, the case history is the modern form of fairy story.

Goodness is unfortunately not enough. Even setting aside its lack of argument or evidence, Mahoney's book is extremely pretentious. 'Thinking' becomes 'mentation', 'discussion' 'dialogue', and 'view' 'perspective': all of these words are of course usually prefaced by 'ongoing'. If scholarship were evaluated by the ratio of references to text, Mahoney would be the world's greatest scholar: even the most obvious statements are often supported by up to 40 references.

I hope I have not misjudged this book. There are after all five extremely glowing encomia on the back cover: but then they are all from psychotherapists. I rest my case. □

Stuart Sutherland is in the Department of Experimental Psychology, University of Sussex, Sussex House, Falmer, Brighton. BN1 9QG, UK.

GOOD RESEARCH NEEDS GOOD *Methods*



Methods
A Companion to Methods in Enzymology
Editors-in-Chief
John Abelson
Melvin I. Simon
ACADEMIC PRESS, INC.
Harcourt Brace Jovanovich, Publishers
San Diego New York Boston London Sydney Tokyo Toronto

Technical innovation provides the infrastructure that sustains rapid growth in biology and biochemistry.

The power of new methods to generate information is striking.

Editors-in-Chief
John N. Abelson
and **Melvin I. Simon**
California Institute of Technology
Pasadena

Each issue of METHODS provides

- An introduction to a rapidly developing area
- Explanations of new approaches being used
- The expertise of the guest editor(s), active in technical innovation in a specific area
- Articles on "how to do it," from those who have done it
- Information allowing all of the techniques discussed to be performed in any modern laboratory

Recently covered topics include:

Protein and Nucleic Acid Crystallization

Edited by **Charles W. Carter, Jr.**
(Vol. 1, No. 1)

The availability of cloned genes has established a powerful partnership between protein chemistry and structural molecular biology. There are important reasons for protein chemists, enzymologists, and molecular biologists to crystallize the molecules they study: answering the questions posed by new proteins and nucleic acids depends critically on knowledge of their three-dimensional structures; X-ray diffraction technology is commonplace and structure determination is no longer necessarily a bottleneck; and important advances have been made in the area of protein and nucleic acid crystal growth itself.

Pulsed-Field Gel Electrophoresis

Edited by **Bruce Birren** and **Eric Lai**
(Vol. 1, No. 2)

The development of gel electrophoresis for separating DNA by size has helped revolutionize the biological sciences. As a result, pulsed-field gel electrophoresis has assumed a critical role in modern biology.

Covalent Modification of Proteins by Lipids

Edited by **Patrick J. Casey**
(Vol. 1, No. 3)

The covalent addition of lipids and lipid-derived groups to proteins is becoming recognized as a mechanism for regulating the localization and function of an increasingly diverse group of these macromolecules.

Forthcoming issues:

- DNA Sequencing, **Bruce Roe**
- Polymerase Chain Reaction, **Norman Arnheim**
- Two-Dimensional Gels, **Michael G. Harrington**
- New Methods in Ligand Identification, **Larry Gold** and **Craig Tuerk**
- Cell Separation and Analysis, **Ellen Rothenberg** and **Rochelle A. Diamond**
- New Methods for Antibody Production, **Richard Lerner** and **Dennis Burton**

Each of these informative editions is available for single-issue purchase.

Cost per issue: **$30.00**

Receive each issue of *Methods: A Companion to Methods in Enzymology* on a regular basis by entering your subscription now.

Volume 1 (1990), 3 issues	In the U.S.A. and Canada: $66.00 All other countries: $84.00
Volumes 2–3 (1991), 6 issues	In the U.S.A. and Canada: $132.00 All other countries: $168.00

ISSN 1046-2023

To enter your subscription or for more information, please write or call:

ACADEMIC PRESS, INC., Journal Promotion Department
1250 Sixth Avenue, San Diego, CA 92101, U.S.A. (619) 699-6742

All prices are in U.S. dollars and are subject to change without notice.

S1178

Reader Service No.39

PLEASE follow these guidelines so that your manuscript may be handled expeditiously.

Nature is an international journal covering all the sciences. Contributors should therefore bear in mind those readers who work in other fields and those for whom English is a second language, and write clearly and simply, avoiding unnecessary technical terminology. Space in the journal is limited, making competition for publication severe. Brevity is highly valued. One printed page of *Nature*, without interruptions of the text, contains about 1,300 words.

Manuscripts are selected for publication according to editorial assessment of their suitability and reports from independent referees. They can be sent to London or Washington and should be addressed to the Editor. Manuscripts may be dealt with in either office, depending on the subject matter, and will where necessary be sent between offices by overnight courier. All manuscripts are acknowledged on receipt but fewer than half are sent for review. Those that are not reviewed are returned as rapidly as possible so that they may be submitted elsewhere without delay. Contributors may suggest reviewers; limited requests for the exclusion of specific reviewers are usually heeded. Manuscripts are usually sent to two or three reviewers, who are chosen for their expertise rather than their geographical location. Manuscripts accepted for publication are typeset from the London office.

Nature requests authors to deposit sequence and crystallographic data in the databases that exist for this purpose, and to mention availability of these data.

Once a manuscript is accepted for publication, contributors will receive galley proofs in about 4 weeks. *Nature*'s staff will edit manuscripts with a view to brevity and clarity, so contributors should check their proofs carefully. Manuscripts are generally published 2–3 weeks after receipt of corrected proofs. *Nature* does not exact page charges. Contributors receive a reprint order form with their proofs; reprint orders are processed after the manuscript is published and payment received.

Categories of paper

Review articles survey recent developments in a field. Most are commissioned, but suggestions are welcome in the form of a one-page synopsis addressed to the Reviews Coordinator. Length is negotiable in advance.

Articles are research reports whose conclusions are of general interest and which are sufficiently rounded to be a substantial advance in understanding. They should not have more than 3,000 words of text (not including figure legends) or more than six display items (figures and tables) and should not occupy more than five pages of *Nature*.

Articles start with a heading of 50–80 words written to advertise their content in general terms, to which editors will pay particular attention. The heading does not usually contain numbers, abbreviations or measurements. The introduction to the study is contained in the first two or three paragraphs of the article, which also briefly summarize its results and implications. Articles have fewer than 50 references and may contain a few subheadings of two or three words.

Letters are short reports of outstanding novel findings whose implications are general and important enough to be of interest to those outside the field. Letters should have 1,000 or fewer words of text and four or fewer display items. The first paragraph describes, in not more than 150 words and without the use of abbreviations, the background, rationale and chief conclusions of the study for the particular benefit of non-specialist readers. Letters do not have subheadings and contain fewer than 30 references.

Commentary articles deal with issues in, or arising from, research that are also of interest to readers outside research. Some are commissioned but suggestions can be made to the commentary editor in the form of a one-page synopsis. Commentaries are normally between one and four pages of *Nature*.

News and Views articles inform non-specialist readers about new scientific advances, sometimes in the form of a conference report. Most are commissioned but proposals can be made in advance to the News and Views editor.

Scientific Correspondence is for discussion of topical scientific matters, including those published in *Nature*, and for miscellaneous contributions. Priority is given to letters of fewer than 500 words and 5 references.

Preparation of manuscripts

All manuscripts should be typed, double-spaced, on one side of the paper only. An original and three copies are required, each accompanied by artwork. If photographs are included, four sets of originals are required; for line drawings, one set of originals and three good-quality photocopies are acceptable. Reference lists, figure legends and tables should all be on separate sheets, all of which should be double-spaced and numbered. Relevant manuscripts in press or submitted for publication elsewhere should be included with each copy of a submitted manuscript, and clearly marked as such. Revised and resubmitted manuscripts should also be clearly marked as such and labelled with their manuscript numbers.

Titles say what the paper is about with the minimum of technical terminology and in fewer than 80 characters in the case of Articles and Letters. Active verbs, numerical values, abbreviations and punctuation are to be avoided. Titles should contain one or two key words for indexing purposes.

Artwork should be marked individually and clearly with the author's name and, when known, the manuscript number. Ideally, no figure should be larger than 28 by 22 cm. Figures with several parts are to be avoided and are permitted only if the parts are closely related, either experimentally or logically. Unlettered originals of photographs should be provided. Original artwork is returned when a manuscript cannot be published.

Protein/nucleotide sequences should ideally be in the three-letter and not the single-letter code for amino acids. One column width of *Nature* can accommodate 20 amino acids or 60 base pairs. Numbering of sequences should be in the left-hand margin only, with a single space between rows.

Suggestions for cover illustrations, with captions and labelled with the manuscript number, are welcome.

Colour artwork. A charge of £500 per page is made as a contribution towards the cost of reproducing colour figures. Inability to pay these costs will not prevent the publication of essential colour figures if the circumstances are explained. Proofs of colour artwork may be sent to contributors under separate cover from their galley proofs.

Figure legends should not exceed 300 words and ideally should be shorter. The figure is described first, then, briefly, the method. Reference to a method published elsewhere is preferable to a full description. Methods are not described in the text.

References are numbered sequentially as they appear in the text, followed by those in tables and finally by those in figure legends. Only papers published or in the press are numbered and included in the reference list. All other forms of reference, including unrefereed abstracts, should be cited in the text as a personal communication, manuscript submitted or in preparation. Text is not included in reference lists. References are abbreviated according to the *World List of Scientific Periodicals* (Butterworths, London, 1963–65). The first and last page numbers are included; reference to books should include publisher, place and date.

Abbreviations, symbols, units and greek letters should be identified the first time they are used. Acronyms should be avoided whenever possible and, if used, defined. Footnotes are not used except for changed addresses.

Acknowledgements are brief; grant and contribution numbers are not allowed.

Submission. Manuscripts can be sent to the Editor at 4 Little Essex Street, London WC2R 3LF, UK or at 1137 National Press Building, Washington, DC 20045, USA. Manuscripts or proofs sent by air courier to London should be declared as 'manuscripts' and 'value $5' to prevent the imposition of import duty and value-added tax (VAT). □

REVIEW ARTICLE

Enzyme catalysis: not different, just better

Jeremy R. Knowles

Where are we in our understanding of enzyme catalysis? The gloomier view is that protein structure and enzyme function are the finely balanced end-products of many weak interactions that can be summed only by massive computing power, and more precise parameterization than we enjoy at present. The cheerier position is that proteins are built on definable principles, and that enzymes use recognizable catalytic devices that will allow us to understand how existing enzymes work and to design new ones. To assess which interpretation is the more realistic, the simple reaction catalysed by triosephosphate isomerase is considered here. This examination illustrates some of the catalytic features of enzymes that are understood, and exposes a few that are not. But overall, the question turns out to have an optimistic answer.

TRIOSEPHOSPHATE isomerase catalyses the chemically simple interconversion of the two triose phosphates, dihydroxyacetone phosphate and *R*-glyceraldehyde 3-phosphate, through the intermediate formation of a *cis*-enediol(ate)[1-3] (Fig. 1). The enzyme merely mediates the protonations and deprotonations necessary to effect two enolizations, one from dihydroxyacetone phosphate to the enediol, and the other from glyceraldehyde 3-phosphate to the enediol. When we compare the catalytic power of the isomerase with that of a simple organic base having a $pK_a \sim 7$ (a notional catalyst from the primordial soup), the isomerase is 10^{10} times more effective[4] (Fig. 2). To improve this primitive catalyst, the kinetic barriers for each of the enolizations must be lowered (Fig. 2, solid arrows), and the enediol(ate) intermediate must be stabilized (Fig. 2, open arrow). How does triosephosphate isomerase do it?

There are, of course, many ways of summarizing the details of an enzyme's active site in terms of devices and motifs, yet in this case a reasonable description of the catalytic apparatus is possible with only three elements: a protein loop that binds and stabilizes the reaction intermediate (fulfilling the open arrow of Fig. 2), and a catalytic base and a catalytic acid to mediate the two enolizations (fulfilling the solid arrows of Fig. 2). What do we know about each of these features?

Intermediate sequestration

Many enzymes have flexible loops, floppy tails, mobile lids, or bivalve-like hinged domains, which appear to close down over the substrate(s) during the chemical changes of the catalytic act[5-10]. Reasons for such sequestration are usually not hard to find: for example, reactive intermediates should not be released into the solvent where they may decompose; stereoelectronics may require a particular substrate conformation to be maintained; water must be excluded; or regiospecificity or stereospecificity demands a tight grip on the reactants. Yet the catalytic value of these conformation changes is often presumed rather than proven.

In triosephosphate isomerase, a loop of ten or eleven amino acids between residues 166 and 176 moves about 7 Å from an 'open' position in the unliganded enzyme into a 'closed' position when there is substrate or inhibitor bound at the active site (Fig. 3*a*)[11-13]. From crystallographic work and molecular dynamics simulations, the conserved residues of this loop (those between residues 168 and 173) seem to form a rigid lid[14] that moves on hinges provided by the unconserved regions on each side. Within the conserved segment, residues 170 to 173 form a looplet that, when the lid is closed, provides a new hydrogen bond to the substrates' phosphate group. As the α carbon of residue 169 is less than 5 Å from that of 174, excision of the four residues of the looplet will not significantly distort the rest of the molecule[15]; but this deletion does make the active site lid too small to envelop the bound substrate (Fig. 3*b*). This mutant protein has been generated, and turns out to have lost nearly 10^5-fold in k_{cat} terms. Substrate binding is not seriously impaired, but the enediol phosphate intermediate analogue, phosphoglycolohydroxamate, is bound much more weakly[15]. It thus appears that the loop closure preferentially stabilizes the enediol phosphate intermediate, as well as the two transition states that flank it (Fig. 2).

Determination of the full energetics of this loopless isomerase confirms that the mutant enzyme has lost its grip on the enediol phosphate intermediate, this failure being vividly evident from the fact that now only one out of six substrate molecules 'makes it' through to product. In the absence of a proper lid, the enediol phosphate intermediate is lost from the truncated enzyme into solution, where it rapidly decomposes (to give methylglyoxal and P_i). (The ingenuity of the enzyme in binding substrate with the phosphate group in the plane of the enediol, so that this decomposition of the intermediate is stereoelectronically disfavoured in the active site, was first noted by Albert Eschenmoser in 1974). The flexible loop of triosephosphate isomerase thus has two catalytic functions: it ensures an efficient throughput of substrate to product, and it stabilizes the reaction intermediate (solid arrow in Fig. 2), which pulls down the free energy of the two flanking transition states. How the loop manages to be so discriminating, in stabilizing the reaction intermediate while affecting the bound substrate and product to a much lesser extent, will have to await a comparison of the high-resolution structures of the enzyme complexed with substrate and with the intermediate analogue phosphoglycolohydroxamate.

Significantly, the loop that we mutilated contains the amino-acid sequence -A-X-G-X-G-K-X-A- (single-letter notation), which has some similarity to the consensus turn that interacts with phosphate groups in some kinases, many dehydrogenases, *ras* p21, and other nucleotide-binding proteins[16-21]. It seems

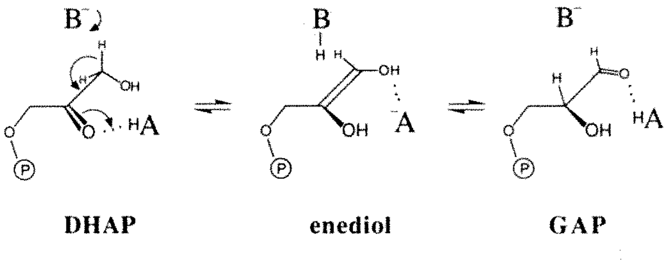


FIG. 1 The reaction catalysed by triosephosphate isomerase. A basic catalytic group B abstracts the pro-*R* proton on C-1 of dihydroxyacetone phosphate (DHAP), assisted by an acidic catalytic group HA, to produce the intermediate enediol. This intermediate then collapses to give the product glyceraldehyde 3-phosphate (GAP) and regenerates the enzyme. The same enediol is produced by the enolization of DHAP or of GAP, and the overall reaction simply puts the two enolizations back-to-back.

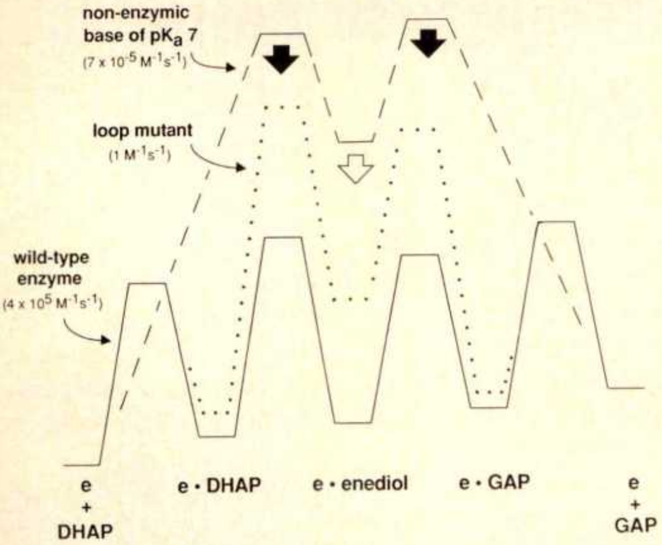


FIG. 2 Free-energy profiles for the isomerization of dihydroxyacetone phosphate (DHAP) and *R*-glyceraldehyde 3-phosphate (GAP), catalysed by wild-type triosephosphate isomerase[45] (solid line), by the loop mutant enzyme that lacks residues 170–173 (ref. 15) (dotted line), or by a non-enzymic base of pK$_a$ ~ 7 (ref. 4) (dashed line). e, Enzyme.

that triosephosphate isomerase has simply recruited, for the catalytic purposes described above, the motif that we have (not altogether frivolously) called the 'phosphate gripper'.

A poised base

From experiments involving kinetics, stereochemistry, chemical modification and X-ray crystallography, we know that the carboxylate of glutamate 165 is the base responsible for the abstraction of the pro-*R* proton of the substrate dihydroxyacetone phosphate (or, for the reverse reaction, the proton at C-2 of *R*-glyceraldehyde 3-phosphate; Fig. 1). From the crystal structures of both the chicken and the yeast isomerases with either the substrate or a substrate analogue at the active site, it is clear that the oxygens of the carboxylate of Glu 165 are beautifully positioned for the proton abstractions required (Fig. 3*c*)[11-13,22,23]. This arrangement is exquisite for four reasons. First, the fit is snug (Fig. 3*c*), the distance from the nearer carboxylate oxygen to the substrate's C-1 or C-2 being only 2.8 or 3.4 Å. Second, the carboxylate group is bidentate, which means that the proton can be removed either from C-1 of dihydroxyacetone phosphate (from left to right in Fig. 1) or from C-2 of glyceraldehyde 3-phosphate (from right to left in Fig. 1), with minimal motion of the catalytic base. Third, the stereoelectronic requirement for rapid enolization[24] (that the abstracted proton lie orthogonal to the enolate plane defined by O-1, C-1, C-2 and O-2; see Fig. 4*a*) is nicely satisfied by the active site arrangement of triosephosphate isomerase. Fourth, the argument of Gandour[25] that a *syn* orbital of a carboxylate ion is much more basic than an *anti* orbital (Fig. 4*b*), has also been recognized in the configuration shown in Fig. 3*c*. So, with all the hindsight and all the physical-organic prejudice available, the choice and position of Glu 165 seem ideal. Indeed, when this group is moved by only ~1 Å (as in the mutant enzyme where Glu 165 is changed to Asp, for which the crystal structure shows no other significant alterations; E. Lolis and G. Petsko, unpublished results), the catalytic power of the enzyme falls by nearly a thousandfold[26]. Although it is too early to generalize, it is evident that in this case at least, the positioning of functionality at the active site of the enzyme needs to be quite precise if full catalytic potency is to be realized. Finally, as has been shown for a number of enzymes, complete removal of the catalytic group and its presumed replacement by water (as when Glu 165 is substituted by alanine or glycine) cuts the catalytic activity by more than a million times (K. Liu, unpublished work). The good news for catalyst engineers is that proper placement of appropriate groups in the right environment seems to be enough. The not-so-good news is that this placement must be very precise. (In this regard, it has been argued by several

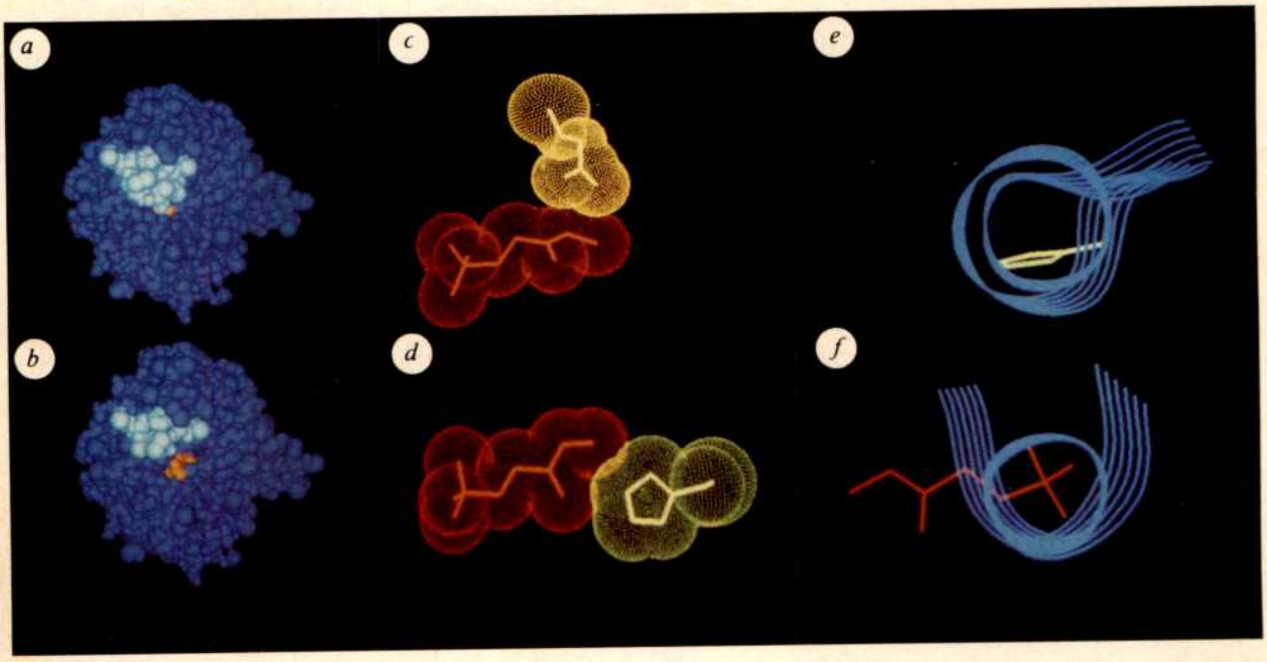


FIG. 3 Catalytic elements of triosephosphate isomerase. *a*, The substrate (orange) is sequestered from solvent by the complete mobile loop (turquoise) (the coordinates are from the crystal structure of the yeast enzyme containing bound phosphoglycolohydroxamate[22,23]); *b*, the substrate (orange) in the same position as in *a*, cannot be sequestered by a loop (turquoise) from which four amino acids (residues 170–173) have been removed (structure from *a*, after emendation and energy minimization); *c*, shows the snug fit of substrate (red) and the catalytic base of Glu 165 (yellow) (the dot surfaces are at the van der Waals' radii); *d*, shows the snug fit of substrate (red) and the catalytic electrophile of His 95 (green) (the dot surfaces are at the van der Waals' radii); *e*, showing the α helix (blue) that is aimed at the imidazole ring of His 95 (green); and *f*, shows the α helix (blue) that is aimed at the phosphate group of the substrate analogue phosphoglycolohydroxamate (red).

authors that the catalytic potential of the carboxylate group has rarely, if ever, been realized in model systems, because of the failure to exploit the *syn* orbitals of this group [27,28].)

An unexpected electrophile

Early experiments indicated that triosephosphate isomerase also uses electrophilic catalysis to speed the enolization steps, for the carbonyl group of the substrate is considerably polarized on binding to the enzyme[29,30]. A likely candidate for this electrophilic role is histidine 95, the Nε of which lies within 2.9 Å of each of the substrate oxygens on C-1 and C-2 (Fig. 3*d*). As with the catalytic base (the carboxyl group of Glu 165), the position of the imidazole ring of His 95 with respect to the substrate seems particularly appropriate, and provides for a strong hydrogen bond to the carbonyl oxygen of either of the substrates dihydroxyacetone phosphate or glyceraldehyde phosphate. There is even the seductive possibility (originally formulated by Jules Rebek) that the proton transfers are mediated merely by rotation of the appropriate side chain: the bidentate carboxyl group for the manipulation of carbon-bound protons, and the bidentate imidazole group for handling the oxygen-bound protons. Mutagenesis experiments have confirmed the importance of His 95 (conversion of this residue to glutamine gives an enzyme that is 100 times less active; conversion to asparagine cuts the activity by 10^4; refs 31-32), and the electrophilic and proton transfer roles of the imidazole ring have been further illuminated by infrared and X-ray crystallographic work on these mutant enzymes[33]. Indeed, when glutamine replaces His 95, the enzyme—deprived of its catalytic electrophile—finds a subtly different mechanistic pathway to achieve the interconversion of the two substrates at 1% of the wild-type rate[31]. This mutant apparently uses Glu 165 opportunistically to effect all the proton transfers, namely those to and from both carbon and oxygen centres.

On this basis, the reader would be forgiven for concluding that Fig. 3*c* and *d* adequately describes a precisely positioned general base (the carboxylate of Glu 165) and a well-placed general acid (the imidazolium of His 95), which are poised for 'push-pull' catalysis. Yet this is not how things are, and our understanding is not complete. The crystal structure of the isomerase indicates from the pattern of hydrogen bonds at the active site[11-13] that the imidazole ring of His 95 is not protonated at neutral pH; this implies that the enzyme uses imidazole rather than the much more electrophilic imidazolium as its acid component. We have investigated this using ^{15}N NMR, which is particularly suitable for such purposes[34]. First, all histidines in the yeast enzyme except that at position 95 were replaced with glutamine, and the resulting (fully active) enzyme was isolated, selectively enriched with ^{15}N-histidine. Using ^{15}N NMR, we found that the pK_a of His 95 is lowered from 6.7 in the denatured protein to less than 4.5 in the native enzyme, and we observed the formation of a new hydrogen bond (presumably to substrate) from the neutral imidazole ring (P. Lodi, unpublished), indicating that the enzyme does use the less acidic neutral imidazole as its electrophile. Why, when everything else about this enzyme seems chemically so reasonable, it should fail to use the more powerful electrophile, remains a puzzle. Yet, so as not to end in a minor key, let us look at one other feature of the active site.

Well-aimed helices

Hol has published a splendid review[35] in which he develops the idea that the formal dipole associated with a classical α helix modulates the properties of groups at the helix termini. Whether the observed effects are due to the electric field of a helix dipole or merely to the local fields of the unsatisfied carbonyl groups and —NH— groups at each end of the helix[36], is less important than the effectiveness of the device, which is well established[37,38]. Triosephosphate isomerase has two conserved α helices that are directed towards the active site. One helix is trained on His 95, the imidazole ring of which is at the positive end (Fig. 3*e*). The aim is precise and, even if we cannot understand why the enzyme should want to lower the pK_a of His 95 to less than 4.5 and so ensure a neutral imidazole, the arrangement of this stretch of helix accounts for the perturbation very well.

The second helix that points into the active site has its positive

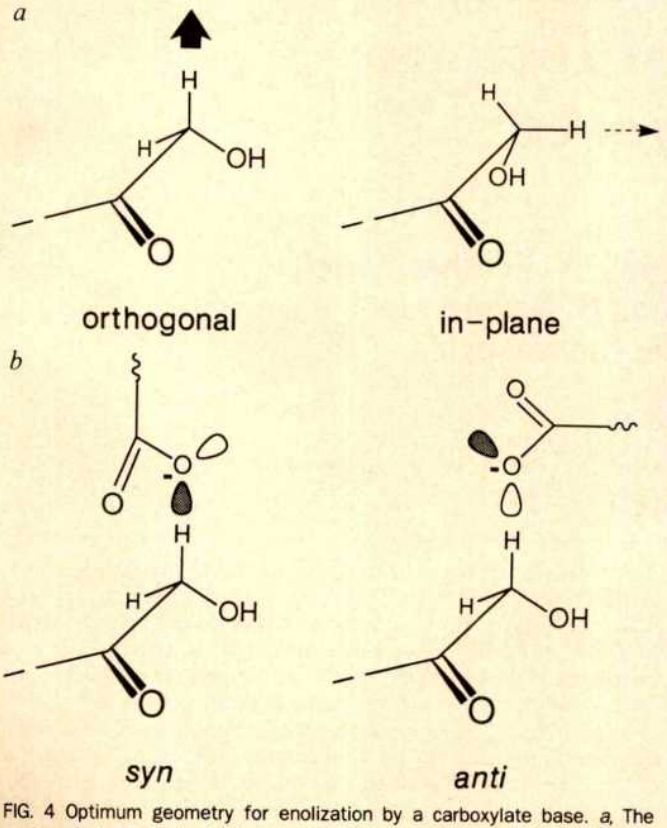


FIG. 4 Optimum geometry for enolization by a carboxylate base. *a*, The proton abstracted should lie orthogonal to the plane of the enolate[24]; and *b*, a carboxylate *syn* orbital (shaded) is believed to be more basic than an *anti* orbital (open)[25].

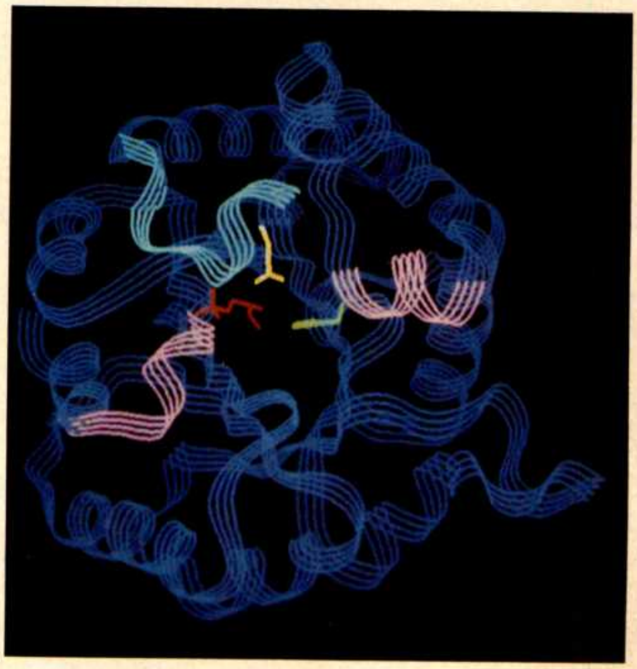


FIG. 5 One subunit of triosephosphate isomerase, with bound substrate (red), Glu 165 (yellow), His 95 (green), the two α helices that are aimed at the active site (pink), and the mobile loop that closes over the bound substrate (light blue).

end aimed at the phosphate group of the substrates (Fig. 3*f*). This arrangement too, is attractive, because there is only one positively charged group at the active site (Lys 12), and yet the substrates bind as their phosphate di-anions[39]. The arrangement shown in Fig. 3*f* provides two hydrogen bonds from main chain —NH— groups (of Gly 232 and Gly 233) to complete the neutralization of charge on the phosphate group.

If a single message emerges after all of the mechanistic and structural scrutiny of triosephosphate isomerase, it is one of precision[40], most evident from Fig. 3*c*–*f*. That the fit of substrate (or, more strictly, transition state[41–43]) to active site is exact has been clear for nearly a century[44], but there are other elements of precision that perhaps we have not fully appreciated. Indeed, one may speculate that the relatively large size of enzymes (compared with most of their substrates) may derive from the need for a matrix that positions their functional groups, focuses their helices and anchors the ends of their mobile loops (Fig. 5). And if our bioorganic effort and chemical mimicry continue to fall short of the catalytic potency of today's enzymes, at least we know that nature has been adjusting and refining for rather longer than we have[45]. ☐

Jeremy R. Knowles is in the Departments of Chemistry and Biochemistry, Harvard University, Cambridge, Massachusetts 02138, USA.

1. Rieder, S. V. & Rose, I. A. *J. biol. Chem.* **234,** 1007–1010 (1959).
2. Rose, I. A. *Brookhaven Symp. Biol.* **15,** 293–309 (1962).
3. Maister, S. G., Pett, C. P., Albery, W. J. & Knowles, J. R. *Biochemistry* **15,** 5607–5612 (1976).
4. Richard, J. P. *J. Am. chem. Soc.* **106,** 4926–4936 (1984).
5. Adams, M. J. *et al. Proc. natn. Acad. Sci. U.S.A.* **70,** 1968–1972 (1973).
6. Clarke, A. R. *et al. Nature* **324,** 699–702 (1986).
7. Ludwig, M. L. *et al. Proc. natn. Acad. Sci. U.S.A.* **57,** 511–514 (1967).
8. Bennett, W. S. Jr & Steitz, T. A. *Proc. natn. Acad. Sci. U.S.A.* **75,** 4848–4852 (1978).
9. Pickover, C. A., McKay, D. B., Engelman, D. M. & Steitz, T. A. *J. biol. Chem.* **254,** 11323–11329 (1979).
10. Winn, S. I., Watson, H. C., Harkins, R. N. & Fothergill, L. A. *Phil. Trans. R. Soc.* **B293,** 121–130 (1981).
11. Banner, D. W. *et al. Nature* **255,** 609–614 (1976).
12. Alber, T. C. *et al. Phil. Trans. R. Soc.* **B293,** 159–171 (1981).
13. Lolis, E. & Petsko, G. A. *Biochemistry* **29,** 6619–6625 (1990).
14. Joseph, D., Petsko, G. A. & Karplus, M. *Science* **249,** 1425–1428 (1990).
15. Pompliano, D. L., Peyman, A & Knowles, J. R. *Biochemistry* **29,** 3186–3194 (1990).
16. Möller, W. & Amons, R. *FEBS Lett.* **186,** 1–7 (1985).
17. Wierenga, R. K., Terpstra, P & Hol, G. J. *J. molec. Biol.* **187,** 101–107 (1986).
18. Seeburg, P. H., Colby, W. W., Capon, D. H., Goeddel, D. V. & Levinson, A. D. *Nature* **312,** 71–75 (1984).
19. Reinstein, J., Brune, M. & Wittinghofer, A. *Biochemistry* **27,** 4712–4720 (1988).
20. Redfield, A. G. & Papastavros, M. Z. *Biochemistry* **29,** 3509–3514 (1990).
21. Saraste, M., Sibbald, P. R. & Wittinghofer, A. *Trends biol. Sci.* **15,** 430–434 (1990).
22. Davenport, R. C. thesis, MIT, Cambridge, Massachusetts (1986).
23. Alber, T. C. *et al. Cold Spring Harbor Symp. quant. Biol.* **52,** 603–613 (1987).
24. Corey, E. J. & Sneen, R. A. *J. Am. chem. Soc.* **78,** 6269–6278 (1956).
25. Gandour, R. D. *Bioorg. Chem.* **10,** 169–176 (1987).
26. Raines, R. T., Sutton, E. L., Straus, D. R., Gilbert, W. & Knowles, J. R. *Biochemistry* **25,** 7142–7154 (1986).
27. Zimmerman, S. C. & Cramer, K. D. *J. Am. chem. Soc.* **110,** 5906–5908 (1988).
28. Huff, J. B., Askew, B., Duff, R. J. & Rebek, J. *J. Am. chem. Soc.* **110,** 5908–5909 (1988).
29. Webb, M. R. & Knowles, J. R. *Biochem. J.* **141,** 589–592 (1974).
30. Belasco, J. G. & Knowles, J. R. *Biochemistry* **19,** 472–477 (1980).
31. Nickbarg, E. B., Davenport, R. C., Petsko, G. A. & Knowles, J. R. *Biochemistry* **27,** 5948–5960 (1988).
32. Blacklow, S. C. & Knowles, J. R. *Biochemistry* **29,** 4099–4108 (1990).
33. Komives, E. A. *et al. Biochemistry* (in the press).
34. Bachovchin, W. W. *Biochemistry* **25,** 7751–7759 (1986).
35. Hol, W. G. J. *Prog. biophys. molec. Biol.* **45,** 149–195 (1985).
36. Tidor, B. & Karplus, M. *Biochemistry* (in the press).
37. Perutz, M. F., Gronenborn, A. M., Clore, G. M. Fogg, J. H. & Shih, D. T.-b. *J. molec. Biol.* **183,** 491–498 (1985).
38. Sali, D., Bycroft, M. & Fersht, A. R. *Nature* **335,** 740–743 (1988).
39. Belasco, J. G., Herlihy, J. M. & Knowles, J. R. *Biochemistry* **17,** 2971–2978 (1978).
40. Blow, D. M. & Smith, J. M. *Phil. Trans. R. Soc.* **B272,** 87–97 (1975).
41. Haldane, J. B. S. in *Enzymes* 182 (Longmans, London, 1930).
42. Pauling, L. *Chem. Eng. News* **24,** 1375–1377 (1946).
43. Leatherbarrow, R. J., Fersht, A. R. & Winter, G. *Proc. natn. Acad. Sci. U.S.A.* **82,** 7840–7844 (1985).
44. Fischer, E. *Ber. dtsch chem. Ges.* **27,** 2985–2993 (1894).
45. Albery, W. J. & Knowles, J. R. *Biochemistry* **15,** 5627–5631 (1976).

ACKNOWLEDGEMENTS. I thank, among many collaborators on the isomerase problem, R. T. Raines, A. Peyman, D. L. Pompliano, E. A. Komives, K. Liu, P. Lodi, E. Lolis and G. Petsko, upon whose contributions much of this account is based. Computer graphics were by P. Lodi and D. Pompliano. Supported by the General Medical Sciences Institute of the NIH and by the NSF.

ARTICLES

Geodetic determination of tectonic deformation in central Greece from 1900 to 1988

H. Billiris[*], D. Paradissis[*], G. Veis[*], P. England[†], W. Featherstone[†], B. Parsons[†], P. Cross[‡], P. Rands[‡], M. Rayson[‡], P. Sellers[‡], V. Ashkenazi[§], M. Davison[§], J. Jackson[||] & N. Ambraseys[¶]

* Department of Surveying, National Technical University, Athens 15773, Greece
† Department of Earth Sciences, University of Oxford, Parks Road, Oxford OX1 3PR, UK
‡ Department of Surveying, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU, UK
§ Institute of Engineering Surveying and Space Geodesy, University of Nottingham, University Park, Nottingham NG7 2RD, UK
|| Department of Earth Sciences, University of Cambridge, Downing Street, Cambridge CB2 3EQ, UK
¶ Department of Civil Engineering, Imperial College, Imperial College Road, London SW7 2BU, UK

The Global Positioning System has been used to measure the relative displacements of fifteen monuments in a hundred-year-old triangulation network spanning part of the Aegean extensional basin. These displacements reflect the tectonic deformation of the region over the past century, showing more than one metre of north–south extension across the network. The crust in this region appears to contain a few slowly deforming blocks separated by more rapidly deforming zones.

AT the same time that plate tectonics was being used successfully to describe a wide variety of geological observations, it was realized that the axioms of plate tectonics do not apply to parts of the continents (see, for example, ref. 1). Deformation is distributed over wide areas on the continents, rather than being concentrated on narrow boundaries as in the oceans. A key aim of tectonics is to determine the most appropriate and economical description of this distributed deformation; without such a description, it is difficult to make progress on studies of the dynamics of the deformation (for example, ref. 2). Most of the quantitative data on active continental deformation come from earthquake seismology and satellite imagery[3–6], but this approach is limited. Earthquake focal mechanisms cannot provide information about rotations, so only the symmetrical part of the deformation field (the strain) can be determined from the seismology; the magnitude of the strain estimated from earthquakes depends on their scalar moments and, for earthquakes in the early part of this century, these are uncertain by about a factor of two; finally, it is known that, in some areas, a large fraction of the deformation occurs without giving rise to earthquakes[5].

For these reasons, it is desirable to form an independent estimate of the rates of deformation in regions of distributed continental deformation. Such estimates can come from geodesy. If benchmarks are implanted in bedrock and their positions are determined precisely at suitably spaced intervals of time, changes in their relative positions may be measured, and hence crustal deformations can be inferred. The recent development of Global Positioning System (GPS) of navigation yields, as a by-product, a tool well suited for such precise positioning measurements.

GPS is based on the simultaneous determination of the distances between a ground receiver and a number of (usually four or more) satellites. In most applications these distances are determined by measuring the time of travel of packets of binary code that modulate two carrier waves that are emitted by the satellites with frequencies of 1,575.42 and 1,227.60 MHz (wavelengths of about 20 and 25 cm respectively). These packets of code cover many cycles of the carrier waves and carry data that allow the immediate determination of position with accuracies of around 100 m and 10 m (for civilian and military users respectively). Geodesists have found, however, that by measuring the phase of the signal (with an accuracy of about 1°, equivalent to about a millimetre), and by using what are essentially interferometric techniques, much higher relative positioning accuracies can be achieved. The technique suffers from several sources of uncertainty (see below), which make millimetric accuracy a rare achievement, but in favourable circumstances GPS is now capable of determining relative positions with centimetric accuracy over distances as great as 1,000 km (see, for example, refs 7, 8).

GPS is particularly attractive because it gives results in a stable global coordinate system (currently the World Geodetic System 1984—WGS84) and so all elements of the displacement gradient tensor are potentially estimable, including the rotation elements that are inaccessible to seismology.

In practice, deformation of the Earth's surface is so slow that several years must elapse before crustal movements (the signal) can reliably be distinguished from measurement uncertainties (the noise). Fortunately, in the course of map making, surveyors have covered many parts of the globe, including some that are tectonically active, with classical triangulation networks. In a few cases the original measurements are published and are of high quality, the original benchmarks still exist, and the network is sufficiently old that there has been a resolvable strain since it was installed. In these cases, it is possible to obtain an immediate determination of crustal motions that took place over decades or more.

Between 1890 and 1900 a first-order triangulation network was established across an area that covers what is now Southern and Central Greece (Fig. 1)[9]. Several of the monuments from the 1890s network have survived, protected in part by their remote locations. In September 1988 we re-occupied fifteen of the surviving sites using GPS receivers (Fig. 2) and were able to determine the relative displacements of those sites over the intervening ninety years. Because the seismic activity of the region over the same time interval has been thoroughly studied[5,10,11], it is possible to compare directly the geodetic and seismological estimates of relative displacement.

Coordinates of the 1988 GPS network

In addition to the fifteen stations of the 1890s network, two satellite-laser-ranging (SLR) sites of the Wegener/MEDLAS project, at Chrissokellaria (XR) and Dionysos (DI) (Fig. 1), were also included in the GPS network. Observations were made with 9 dual-frequency TI-4100 receivers over an 8-day period, of which seven were observation days, one instrument remaining at Dionysos throughout the survey. Each station was observed for at least two days, and successive days of observation had at least four stations in common.

GPS phase observations, and their processing, suffer from a number of sources of error, of which the most significant are probably those resulting from refraction of the signals in the ionosphere and troposphere, from imprecise knowledge of the positions of the satellites, and from the receivers' losing track of the phase of the incoming signals because of physical obstruction or electrical interference.

We made measurements of phase on the two GPS carrier frequencies and corrected for the influence of ionospheric refraction by the conventional means, which take advantage of the fact that the phase delay in the ionosphere is inversely proportional to the frequency of the signal. We could not correct for refraction in the troposphere because we had no reliable data

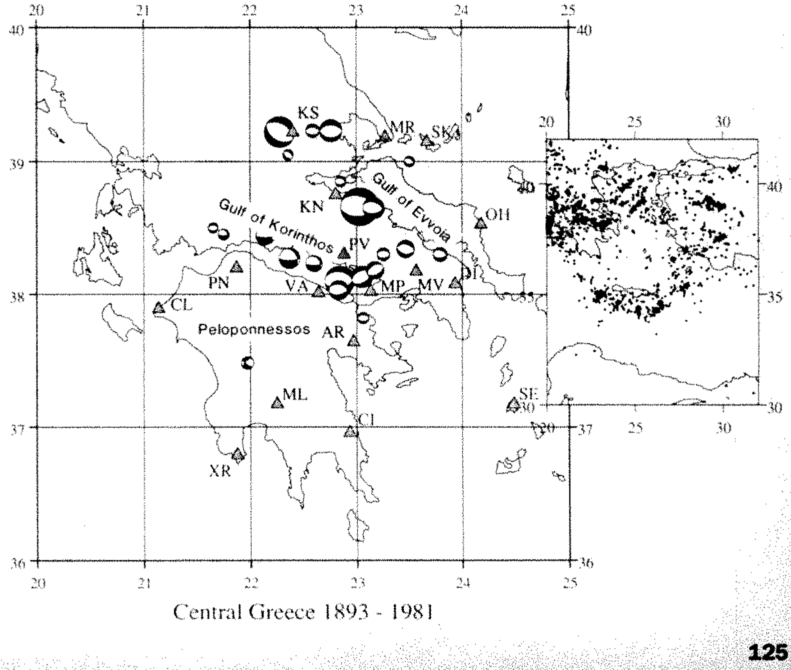


FIG. 1 Locations of the sites in the geodetic network discussed here, and fault-plane solutions for all earthquakes with magnitude $M_S \geq 5.8$ that occurred between 1890 and 1988 within that network[11]. Fault-plane solutions were obtained from teleseismic waveform observations where possible; failing this, the strike, dip and rake were estimated from surface faulting associated with the earthquake. In the absence of these direct observations, the fault-plane solutions of nearby events, or the orientation of nearby active faulting, were used. The areas of the circles containing the fault-plane solution are proportional to the seismic moments of the earthquakes. Triangles denote sites common to the 1890s and 1988 networks as well as the two SLR sites of the 1988 network. The sites are labelled with abbreviations: KS, Kassidiaris; MR, Makra Rachi; SK, Skopelos; KN, Knimis; OH, Ochtonia; PV, Palaiovuna; MV, Megalo Vuno; DI, Dionysos SLR station; PN, Panachaikon; VA, Valtsa; MP, Makriplaji; CL, Chlemutsi; AR, Arachneon; XR, Chrissokellaria SLR station; ML, Malevon; CI, Chionovuni; SE, Serifos. Inset shows the regional setting; small dots give the locations of earthquakes with $m_b > 5$ for the map area taken from the USGS Preliminary Determination of Epicenters.

on the amount of water in the atmosphere, such as could be provided by weather balloons or water-vapour radiometers. Consequently, uncertainty at the one- or two-centimetre level remains from this source. In our analysis, we have used positions of the GPS satellites as given by their own broadcast estimates of their orbits. Orbit errors of about 20 metres lead to scale uncertainties of around 1 part in 10^6. These errors may be reduced either by employing the more precise orbits released later by the US Department of Defense, or by constraining the orbits with GPS phase data collected (at the same time as the survey) at stations of known position. The 1988 coordinates of our network will be re-determined later using precise orbits, but the coordinates reported here are based on the broadcast orbits and so have uncertainties of one or two centimetres from this source.

Because of the complexities in treating the data, it has become standard geodetic practice to process important GPS campaigns with more than one software package. Our data were processed by two independent methods.

The first method used the Bernese V3.1 package[12]. The data were processed in seven sets, corresponding to the seven days of observation. The solution for each day yielded, for each station observed that day, three-dimensional cartesian coordinates in the WGS84 system. These coordinates were derived as differences from the position of the Dionysos SLR station. An estimate was obtained for the covariance of the coordinates. The seven days of data (coordinate differences and their covariance matrices) were adjusted[13] to provide a single estimate for the coordinates of all points, based on the fixed coordinates of Dionysos. A data validation was then carried out that compared the coordinate differences obtained from the three-dimensional adjustment with those generated by the separate daily solutions. The process involved testing based on the tau (τ) distribution[14] and produced the conclusion that there were no significant inconsistencies in the data. The standard deviations of the results, expressed in terms of their horizontal components, are 0.03 m in latitude and 0.04 m in longitude, with respective maximum values of 0.06 m and 0.07 m.

An analysis was also carried out using the MAGNET-101 V7.21 software[15]. Although this program is not designed for very-high-precision applications, it was considered adequate to afford an independent confirmation that the results do not contain errors that would invalidate our analysis of the horizontal strain. Again, the analysis involved two stages. In the first, individual baselines and their covariances were determined from the MAGNET software, and in the second stage they were adjusted to give a single estimate of the coordinates[13]. Initial individual baseline estimates from the MAGNET program were then compared with the adjusted baseline values. Where an individual estimate differed significantly from the adjusted values for that baseline, and there were sufficient additional observations of the same baseline to identify the discrepant estimate as an outlier, it was deleted from the final adjustment. In the final adjustment of the baselines determined using the MAGNET software, there were 135 measurements of a network of 44 baselines, of which 15 were measured only once. The standard deviations of the final results are between 0.02 and 0.04 m. Note that this computation is not rigorous in that (unlike the computation based on the Bernese software) it does not take account of correlation between simultaneously observed baselines.

The Bernese and MAGNET solutions have been compared by computing relative motion vectors of the stations (1890s to 1988) for each solution. The differences in the lengths of the vectors determined by these two methods is small: the maximum difference in length is 0.06 m and the average is around 0.02 m, with the MAGNET solution generally being the longer. The maximum difference in orientation of the relative motion vectors is only 10°, with an average around 3°. These differences are consistent with the uncertainties in position for the Bernese solutions quoted above.

The uncertainties in the Bernese solutions are equivalent to an average horizontal precision of around 0.4 p.p.m. between any pair of stations in the network. Because these coordinates are obtained by the more rigorous procedure, they are the ones referred to as the 1988 coordinates in the discussion below. It must be emphasised, however, that either set of GPS solutions would lead to the same conclusions about the deformation. The GPS results are an order of magnitude more precise than those determined from readjustment of the 1890s observations (below). In turn, the uncertainties in the 1890s coordinates are small compared with the total displacement across the network (Fig. 2).

Coordinates of the 1890–1900 network

The basis for the geodetic determination of the strain of the region is two sets of coordinates for fifteen monuments of the 1890s survey that are separated in time by ninety years. The coordinates of the monuments in September 1988 are expressed in the WGS84 coordinate system. The published observations from the 1890s survey consist entirely of theodolite directions (502 angles involving 93 stations[9]). These are horizontal angles from an arbitrary origin; there is, therefore, no information on the position, orientation or scale of the original network, and assumptions must be made about these before we can compare modern determinations of position with the original observations.

To choose the scale of the original network we make the assumption that the length of a particular baseline in the Peloponnessos (Panachaikon-Chionovuni in Fig. 2*a*) has not changed between the two surveys. This assumption has some independent support from the absence of large earthquakes in the central Peloponnessos (Fig. 1) and seems to be justified *a posteriori* by the small displacements determined for other sites in the Peloponnessos (Fig. 2*a*; also see below). The remaining assumptions, to give orientation and translation of the original network with respect to the modern one, are completely arbitrary. Here we discuss mainly those coordinate differences obtained by assuming that the same pair of stations used to fix the scale (PN and CI) did not move with respect to their modern positions (Fig. 2*a*); but a second, equivalent, representation is shown in Fig. 2*b*.

Positional uncertainties of up to 50 cm were found for the readjustment of the 1890s network, but the typical uncertainty in the area reoccupied by GPS in 1988 was ~10–40 cm. The mean observational standard error for directions was 0.55 seconds of arc.

Horizontal relative displacements 1890–1988

Horizontal relative displacements are calculated from the difference between the coordinates of the 1988 GPS network and those derived from the adjustment of the 1890s network, as described above. Figure 2*a* shows horizontal relative displacements calculated assuming Panachaikon and Chionovuni to be fixed to their 1988 positions. Note that these displacements contain no information on translation or rotation of the network as a whole. To emphasize this point, Fig. 2*b* shows displacements calculated under different assumptions, which are described in the caption.

The assumption made in deriving the scale of the network—that the length of the line joining Panachaikon and Chionovuni did not change between the two surveys—seems to be justified, because the calculated displacements of three other points in the Peloponnessos (Valtsa, Arachnaeon and Malevon) are less than the uncertainties in their original positions (about ten centimetres). There has, therefore, been no resolvable shear strain of the central Peloponnessos over the past 90 years. (The other point on the Peloponnessos (Chlemutsi) seems to have been displaced by about 70 cm (Fig. 2). The triangulation point is, however, on top of the wall of an old castle; the displacement may be due either to tectonic activity or to settling of the fabric of the castle.) Formally, there could still be an error in the scale of the network, if there had been a purely isotropic dilatation or contraction of the region in the time interval between measurements, but such a strain seems unlikely on geological grounds.

Despite the indeterminacy in translation and rotation of the network as a whole, the quantities of geological interest—the relative displacement gradients (subject to an unknown rigid-body rotation)—can be determined from coordinate differences within the network between the 1890s and the present.

Distibution of deformation

Uncertainties in the original (1890) coordinates are between 10 and 40 cm, and the baselines are 50–150 kilometres long, so strains smaller than about 2×10^{-6} cannot be detected. Figure 3 shows the differences between the horizontal displacements for each pair of points in the network. The shading distinguishes those displacements that are greater than the uncertainties in the original positions from those that are not resolvable, given the uncertainties. There are three regions in which the sites have not moved resolvably with respect to each other. The first group contains five sites within the Peloponnessos: Panachaikon (PN), Valtsa (VA), Arachneon (AR), Chionovuni (CI) and Malevon (ML). The very small (~10 cm) relative displacements between these points are consistent with the absence of major seismic activity in the central Peloponnessos over the last hundred years (Fig. 1). There are, nevertheless, several active normal faults within the Peloponnessos (see, for example, ref. 16). Most of those faults lie close to the edge of or, like that which slipped in the Kalamata earthquake of September 1986 (22° E, 37° N), outside our network. Although there is a report[18] of Sparta being destroyed by an earthquake in the fifth century BC, this event need not have been large, and may also have had its epicentre outside the region covered by our network. It appears from the measurements reported here that the Peloponnessos is being strained at least ten times more slowly than the more seismically active region to the north of the Gulf of Korinthos.

The second group contains four sites immediately north of the Gulf of Korinthos: Palaiovuna (PV), Megalo Vuno (MV), Makriplaji (MP) and Ochtonia (OH). Relative displacements between these points are discernably larger than between those of the previous group (Fig. 3). Unlike the Peloponnessos, this region has experienced several large earthquakes since 1890 (Fig. 1); these could account for displacements of a few tens of centimetres between the stations (see Fig. 1 and Tables 1 and 2 of ref. 11).

The third group lies in the northernmost part of the network. Kassidiaris (KS), Makra Rachi (MR) and Knimis (KN) have not moved resolvably with respect to each other and since 1890 there have been no large earthquakes within the triangle described by these points, although there have been several immediately to the north of this part of our network (Fig. 1).

The displacement of the monument on Serifos with respect to both the Peloponnessos and central Greece is unexpected, given the low level of seismic activity in this part of the Aegean (Fig. 1). Until other 1890s points near Serifos are re-occupied, however, we cannot assess the significance of this displacement.

Comparison with seismological estimates

Approximately 1.1 m of extension has occurred across the network in the 90–100-yr interval between the surveys, as indicated by the displacements of the northernmost sites in Table 1. The portion of the network in which the extension occurs (to

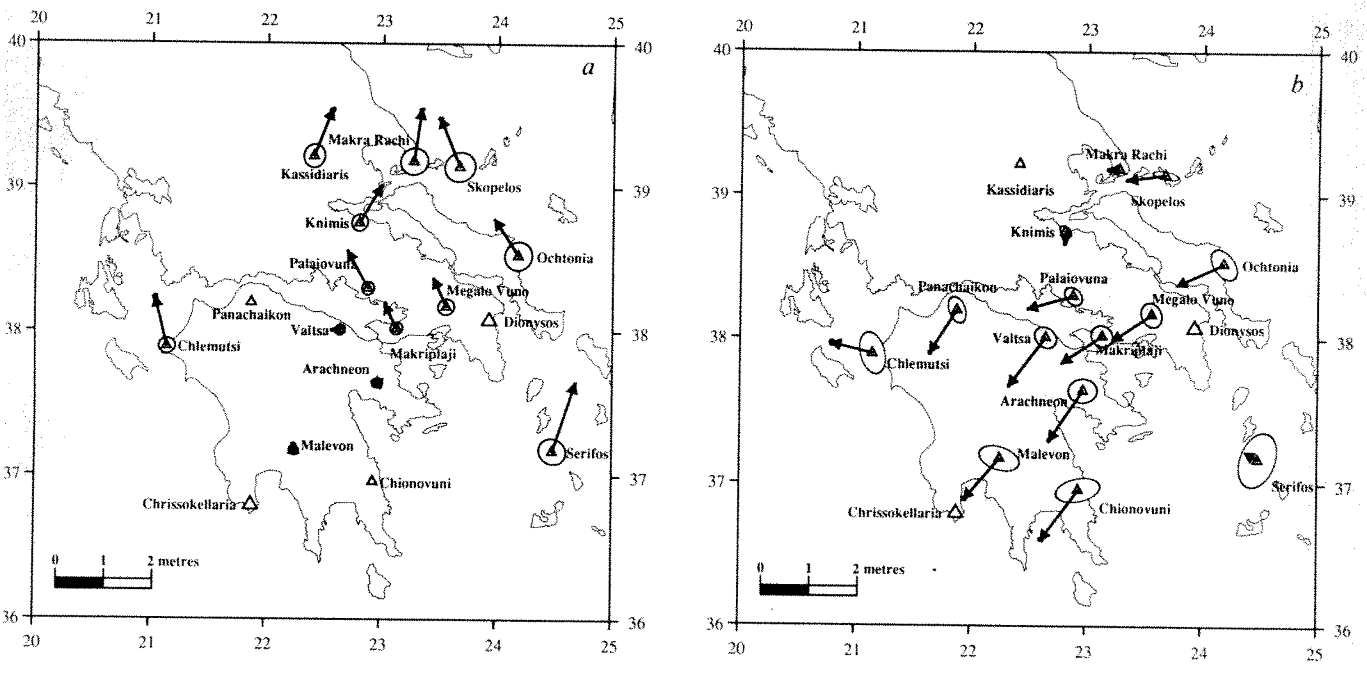


FIG. 2 *a*, Horizontal relative displacements calculated from coordinates derived for the 1890s triangulation network and for the 1988 GPS network, assuming Panachaikon and Chionovuni to be fixed in their 1988 positions. Ellipses centred around the sites (triangles) give the uncertainties associated with the readjustment of the 1980s network. Ellipses corresponding to the positions of the 1988 network are plotted at the head of the displacement vectors but, at the scale used for the displacements, are too small to be clearly visible. Larger triangles, without arrows or ellipses, are (modern) SLR sites of the Wegener/MEDLAS project. The bar at the lower left shows the scale for the arrows and ellipses. *b*, Horizontal relative displacements calculated with orientation, translation and scale given by the following assumptions: the scale is fixed by holding the length of the line between Panachaikon and Chionovuni to its 1988 length; Kassidiaris is held to its 1988 position; and the line joining Kassidiaris to Makra Rachi is held at its 1988 orientation. These conditions reflect the presumption that the most northerly part of the network has been displaced least with respect to Eurasia in the last 100 years. Apart from small differences in coordinates of the 1890s points owing to the different ways that the uncertainties are distributed during the readjustment, these displacements are equivalent to those in *a* with the addition of a translation and a rotation.

the north of the Peloponnessos) is about 120 kilometres across. The extensional strain is therefore $\sim 10^{-5}$, which corresponds to a strain rate of $10^{-7}\ \mathrm{yr}^{-1}$, or $3\times10^{-15}\ \mathrm{s}^{-1}$.

The displacement accounted for by earthquakes over the same time interval has been calculated by Ambraseys and Jackson[11], using the method of Kostrov[16]. The major uncertainties in the calculation lie in the estimates of the scalar moments, M_0, of the earthquakes, which are directly related to the seismic portion of the strain[16]. For most of the earthquakes, M_0 was obtained by using an empirical relationship[11] between M_S, the magnitude of the earthquake, and M_0. Uncertainties of ±0.25 in M_S were used to derive maximum and minimum estimates of the scalar moments. An additional uncertainty arises because the relationship between M_S and M_0 appears to exhibit regional variations[17]. The estimates of displacement from seismicity in Table 1 were obtained using the regional relationship that is suggested (ref. 17; see also ref. 11, Fig. 5) to be more appropriate for the Aegean. For further discussion of these uncertainties see references 1, 3, 4 and 13.

The probable[11] value of the displacement across this region that is accounted for by earthquakes with $M_S > 5.8$ is 45 cm (Table 1). The slip in all earthquakes with $M_S < 5.7$ increases this quantity by about 50% (ref. 11) to about 70 cm. Extreme values of displacement, allowing for systematic biases of ±0.25 in M_S, might be as low as 35 cm or as high as 125 cm. Thus the seismic strain between the 1890s and 1988 appears to be about 60% of the geodetic strain, although it may be as high as the geodetic strain if the seismic moments of the earthquakes have been systematically underestimated[11].

Comparison with other observations

Arguments based on the regional plate tectonic setting of Central Greece suggested to McKenzie[10,19] that the Peloponnessos is moving southwestward with respect to northern Greece. Yet the slip vectors in the earthquakes in central Greece are almost entirely north-south[5]. McKenzie and Jackson[20] suggested that these observations could be accommodated if the deformation in Central Greece—in the region of this network—were a combination of normal faulting and clockwise rotation of crustal blocks that are separated by these faults. The available palaeomagnetic data[21,22] suggest that there has indeed been such clockwise rotation over the past few million years.

We would like to be able to compare the observations of slip vectors and rotations with the geodetic determinations of displacements. Unfortunately there is no information about the original orientation of the network and, unlike the estimation of the scale, there is no testable assumption that we can make to estimate that original orientation. A simple assumption is that the most northerly part of the network has been rotated least with respect to stable Eurasia. Figure 2*b* shows the displacements calculated under this assumption.

TABLE 1 Relative displacements across the network between 1890 and 1988

Site	E-W	N-S	Magnitude
Geodetic displacement (cm)			
Kassidiaris	39.0	95.1	102.8
Makra Rachi	18.4	108.1	109.7
Skopelos	−32.1	103.5	108.4
Seismic displacement (cm)	E-W	N-S	Magnitude
Minimum	0.7	25.4	25.4
Probable	1.2	45.1	45.1
Maximum	2.2	82.5	82.5

Geodetic estimates are from the relative-motion vectors displayed in Fig. 2*a*. Seismic displacements are from ref. 11 (see text). In comparing the two estimates of displacement, it must be remembered that the 1890s triangulation contained no information on orientation, and therefore that only the magnitudes of the displacements are directly comparable.

The general southwesterly movement (Fig. 2*b*) of the Peloponnessos with respect to northern Greece is consistent with McKenzie's[10,19] suggestion. Furthermore, the northwesterly movement of the points immediately north of the Gulf of Korinthos with respect to the Peloponnessos (Fig. 2*a*, *b*) is consistent with a combination of north–south extension and clockwise rotation, as suggested by McKenzie and Jackson[20]. There are, however, too few points in this survey, and they are placed too far apart, for us to determine whether there is pervasive block rotation of the kind envisaged in ref. 20. It seems clear, however, that if the central Peloponnessos is accepted as a single rigid block, it cannot be rotating appreciably with respect to northern Greece.

Conclusion

It is inevitable that, in a geodetic study based on a network almost a century old, there should be uncertainty about the

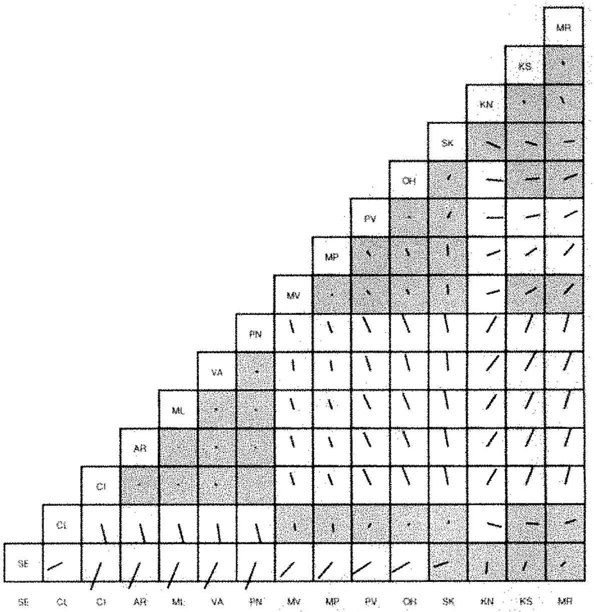


FIG. 3 Each square in the diagram shows the displacement of the station given at the head of the column relative to the station at the left of the row; the vector depicting the displacement has its origin at the centre of the square. Squares are white when the magnitude of the relative displacement is more than 2 standard deviations of the error in the relative displacement, and grey when it is less than this. Groups of grey squares over several neighbouring sites provide an indication of areas that may have moved as rigid blocks over the past 100 years. Abbreviations the same as in Fig. 1.

quality of the original data and monumentation. The adjustment, described above, of the published observations of the 1890s survey demonstrates their high quality. (A standard deviation of 0.55 seconds of arc corresponds to one part in 10^6, which is close to the limit of precision in classical surveys.) The apparent relative horizontal displacements within the central Peloponnessos (Fig. 2*a*) are of the order of 10 cm over baselines 100-150 km long: one part in 10^6 or less. This result suggests that the monuments themselves are stable to within a few centimetres over this timescale, and the apparent motions are probably attributable to the uncertainty in original observations. Possible slumping of ground (or of buildings, as at Chlemutsi), lightning strikes, and acts of war are more serious threats to monument stability in this region than is occasional shaking in earthquakes. We therefore conclude that the relative displacements, by tens of centimetres, of monuments in the more tectonically active part of the network represent (within the uncertainties) true crustal motions.

The observations reported here illustrate both the strengths and the limitations of surveys based on old triangulations. The length of the time interval involved ensures that, in this region at least, the signal is considerably greater than the noise, allowing a coarse-grained picture of the deformation to be obtained. This time interval, furthermore, is comparable with the interval (typically a few hundred years) between earthquakes on the same fault; therefore, the measurement covers a timescale that is close to that of the phenomenon of interest. The price that must be paid is that there is often no information on translation, rigid-body rotation or uniform dilatation of the network. There is also a lower limit on the scale of deformation that can be investigated: the spacing between the points in the original survey is of the order of 60 km—about twice the spacing between the active faults in the central part of the network (Fig. 1). In addition, the 1890 survey—like most classical triangulation networks—carries no precise height determination, so only horizontal relative displacements may be recovered.

The pattern of geodetic strain in central Greece seems to be one of the relative motion of a few rigid blocks, several tens to a hundred kilometres on a side, separated by regions a few tens of kilometres wide in which strain accumulates rapidly. The displacements of PV, MV and MP (Fig. 2) on the north side of the Gulf of Korinthos are about 60 cm with respect to the Peloponnessos. In turn, the four most northerly sites in the network seem to have moved with respect to the sites immediately north of the Gulf of Korinthos by about 40 cm. The two most seismically active parts of the region covered by the network are the Gulfs of Korinthos and Evvoia (Fig. 1), and these are also the most dramatic morphological features of the region, being large gulfs bounded by active normal faults. The geodetic observations are in qualitative agreement with the seismic data, in that the relative displacements within the network can largely be accounted for by movement across these two gulfs.

The only quantitative comparison that we have made between geodetic and seismic observations is in terms of the total displacement across the region. The preferred value for the displacement expressed in earthquakes (~70 cm, Table 1) is about 60% of the geodetically determined displacement. Such close agreement between the seismic and total strain is perhaps surprising in view of the abundant field evidence for aseismic deformation processes in near-surface rocks. It has been suggested previously[6,23] that seismic strain may make up 50% or more of the total strain in some deforming continental belts (including the Aegean area). This study provides independent support for that suggestion from a part of one of these belts.

A final note of caution is necessary. Although the results reported here appear to identify several undeforming regions, these regions all contain faults that seem to have been active during the Quaternary (see ref. 11 for a summary). The appearance of rigidity may thus be the result of our inability to resolve a strain of less than about one part in 10^6. It is perhaps more reasonable to regard these 'blocks' as regions that are deforming about an order of magnitude more slowly than the zones that separate them but which are, nonetheless, accumulating strain that could lead to earthquakes. It seems probable that the greater precision afforded by GPS surveys could eventually yield information about the accumulation of strain in such regions that would be valuable in earthquake hazard assessment. ☐

Received 10 August 1990; accepted 16 January 1991.

1. Molnar, P. *Nature* **355,** 131-137 (1988).
2. England, P. C. & Jackson, J. A. *A. Rev. Earth Sci.* **17,** 197-226 (1989).
3. Molnar, P. & Tapponnier, P. *Science* **189,** 419-426 (1975).
4. Molnar, P. & Deng, Q.-D. *J. geophys. Res.* **89,** 6203-6228 (1984).
5. Jackson, J. A. & McKenzie, D. P., *Geophys. J. R. astr. Soc.* **93,** 45-73 (1988).
6. Ekström, G. & England, P. C. *J. geophys. Res.* **94,** 10231-10258 (1988).
7. Lichten, S. M. & Bertiger, W. I. *Bull Geodes.* **63,** 167-189 (1989).
8. Dixon, T. *et al. Eos* **71,** 1051-1056 (1990).
9. Hartl, H. *Comptes Rendus de la Seizième Conférence Genérale de l'Association Géodésique Internationanale* II (1911).
10. McKenzie, D. P. *Geophys. J. R. astr. Soc.* **30,** 109-185 (1972).
11. Ambraseys, N. N. & Jackson, J. A. *Geophys. J.* **101,** 663-708 (1990).
12. Beuttler, G., Bauerisma, I., Gurtner, W. & Rothacher, M. in *GPS Techniques Applied to Geodesy and Surveying* 363-380 (Springer, Berlin, 1988).
13. Cross, P. A. *Proc. XIX Int. Congress Federation International de Géometre* **5,** 336-348 (FIG, Helsinki, 1990).
14. Pope, A. J. in *NOAA Tech. Rep.* N0565NGS1 (NOAA, Washington, DC, 1976).
15. Hatch, R. & Larsen, K. in *Proc. 1st Int. Symp. on Precise Positioning with GPS*, Rockville 285-297 (National Geodetic Information Center, NOAA, Rockville, Maryland, 1985).
16. Kostrov, V. V. *Izv. Acad. Sci. USSR Phys. Solid Earth* **1,** 23-44 (1974).
17. Ekström, G. & Dziewonski, A. M. *Nature* **333,** 319-323 (1988).
18. Thucydides *History of the Peloponnesian War* (transl. Warner, R.) 94 (Penguin, London 1972).
19. McKenzie, D. P. *Geophys. J. Roy. astr. Soc.* **55,** 217-254 (1978).
20. McKenzie, D. P. & Jackson, J. A. *Earth planet. Sci. Lett.* **65,** 182-202 (1983).
21. Kissel, C., Laj, C. & Mazaud, A. *Geophys. Res. Lett.* **13,** 1446-1449 (1986).
22. Kissel, C. & Laj, C. *Tectonophysics* **146,** 183-201 (1988).
23. Jackson, J. A. & McKenzie, D. P. *Geophys. J. Roy. astr. Soc.* **77,** 185-264 (1984).

ACKNOWLEDGEMENTS. This work was supported by a grant from Oxford University to P.E. and B.P., by the Hellenic National Committee on Geodesy and Geophysics, by Robert Saunders Ltd (mountain tents) and by the Rotal Society. We thank K. Argiros, V. Balis, T. Bickell, S. Felekis, P. Milas, L. Moresi, R. Newman, K. Orphanos, A. Papafitsorou, P. Powell, T. Strange, Ch. Vlachos and S. Watson for assistance in the field. The help of Kim Kastens and the Lamont-Doherty Geological Observatory Aegean Geodetic Project is gratefully acknowledged; we are particularly grateful to Ken Hurst for manning the Dionysos station.

Three-dimensional structure of plant light-harvesting complex determined by electron crystallography

Werner Kühlbrandt & Da Neng Wang

European Molecular Biology Laboratory, Meyerhofstrasse 1, 6900 Heidelberg, Germany

The structure of the light-harvesting chlorophyll *a*/*b*-protein complex, a membrane protein serving as the major antenna of solar energy in plant photosynthesis, has been determined at 6 Å resolution by electron crystallography. Within the complex, three membrane-spanning α helices and 15 chlorophyll molecules are resolved. There is an intramolecular diad relating two of the α helices and some of the chlorophylls. The spacing of the chlorophylls suggests energy transfer by delocalized exciton coupling and Förster mechanisms.

SOLAR energy is collected in photosynthetic membranes of all green plants by the light-harvesting chlorophyll *a*/*b*-protein complex associated with photosystem II (LHC-II). The energy is passed on to the photosynthetic reaction centres I and II where it is used to generate a potential gradient across the membrane. This provides the energy for oxygen evolution, ATP synthesis and, ultimately, carbon fixation.

LHC-II is isolated from chloroplast membranes as a trimer[1] of three identical or nearly identical monomers[2], each consisting of one polypeptide (233 amino acids[3], relative molecular mass of 25,000) and non-covalently attached chromophores (eight chlorophyll *a*, seven chlorophyll *b* (ref. 1) and several carotenoids[4]). Altogether, LHC-II binds ~50% of all chlorophyll on earth. The complex forms large, well-ordered two-dimensional crystals[5] (layer group p321, $a = 127$ Å, $c = 60$ Å). Henderson and Unwin developed a method for determining the structure of biological macromolecules by electron diffraction, electron microscopy and image processing of two-dimensional crystals[6], which has recently been extended to high resolution[7-9]. By this method, the phases of structure factors are determined directly from the Fourier transform of electron micrographs to a resolution that is limited by the finest detail reproduced in the image. Untilted crystals yielded phases to 3.4 Å (ref. 5) whereas image transforms of highly tilted crystals tended to be weak at high resolution in the direction perpendicular to the tilt axis. In this study, data extending to 6 Å resolution were used (Fig. 1). Future analysis at higher resolution should be possible as more phase data from highly tilted two-dimensional crystals are incorporated.

Polypeptide structure

A three-dimensional map of LHC-II (Figs 2 and 3) was generated from 2,737 structure factors in the asymmetric unit. Figure 2 shows the trimeric complex, consisting of three monomers related by crystallographic symmetry. The overall shape of the trimer is roughly cylindrical, with a diameter of 73 Å and a thickness of 60 Å. The space around the perimeter and a central region of triangular cross-section are almost featureless and probably contain unresolved or partly disordered lipid and detergent. A part of the map of roughly elliptical, 30 Å × 50 Å cross-section which we believe to be the LHC-II monomer is shown in Fig. 3. Well resolved features highlighted in colour, are found mainly in the 40 Å-thick central section of the map which presumably coincides with the lipid bilayer.

The most prominent features of the map are three rod-like regions of density (purple) representing three membrane-spanning α helices, labelled A, B and C in Fig. 3. The central helices A and B are inclined by 25° and 31° with respect to the membrane normal and slightly twisted round one another, with a contact distance of about 10 Å. This distance and the included angle of −56° are characteristic for close-packed helices of this polarity in soluble proteins[10]. Helices A and B are ~46 Å and 49 Å long, corresponding to about 31 and 33 residues, respectively. They are thus longer than all membrane-spanning α helices of bacteriorhodopsin[9] and of the bacterial reaction centre[11]. The upper parts of helices A and B are related by approximate, non-crystallographic twofold symmetry. The lower third of helix A but not of helix B is noticeably kinked and almost parallel to the membrane normal. The non-crystallographic twofold symmetry does thus not extend to the lower half of the complex.

Helix C is inclined by 11° with respect to the membrane normal. Its length of about 30 Å, corresponding to 20 residues, is similar to that of the shorter transmembrane helices in the two other membrane proteins[9,11]. This helix probably delineates the hydrophobic core of the lipid bilayer which is 30 Å thick[12]. A side view (Fig. 3*c*) shows that helices A and B extend vertically ~7 and 9 Å beyond the top of helix C. Taking the total bilayer thickness as 40–45 Å (ref. 13) this means that they protrude somewhat beyond the membrane surface. There is no evidence of other polypeptide structures spanning the membrane.

At this contour level, little detail is visible above or below the central 40 Å section, probably due to the lack of distinct secondary structure in this region, or possibly because this part of the polypeptide is disordered. At a lower contour level (not shown), several features appear which we have tentatively assigned to loops connecting the helices and to the amino and carboxy termini of the polypeptide. On the upper side of the complex, two hook-like extensions of helices A and B with the same approximate two-fold symmetry protrude above the membrane surface, linking the two halves of the monomer which appear roughly triangular in projection (Fig. 3*a*). Helix C seems to be connected to the hook on helix A, whereas the hook on helix B ends in a convoluted structure which is not connected to another helix and therefore is likely to represent one of the polypeptide termini. On the other side of the membrane (Fig. 3*b*), helix B seems to be linked to helix C. Two roughly linear regions of density are connected to helix A which may lead to the other polypeptide terminus. The surface on this side of the membrane appears more or less flat.

Three hydrophobic membrane-spanning α helices of about 20 residues each have been predicted by the polypeptide sequence of LHC-II (refs 14, 15). The map shows that helices A and B are considerably longer than expected. The polypeptide sequence revealed an internal homology with 37% sequence identity of residues 47 to 79 and 162 to 194 in the mature protein[16,17]. Since the homology relates equivalent segments of two predicted helices, it seems likely that these domains should be identified with the upper halves of helices A and B and their extensions which are related by twofold symmetry. The positions

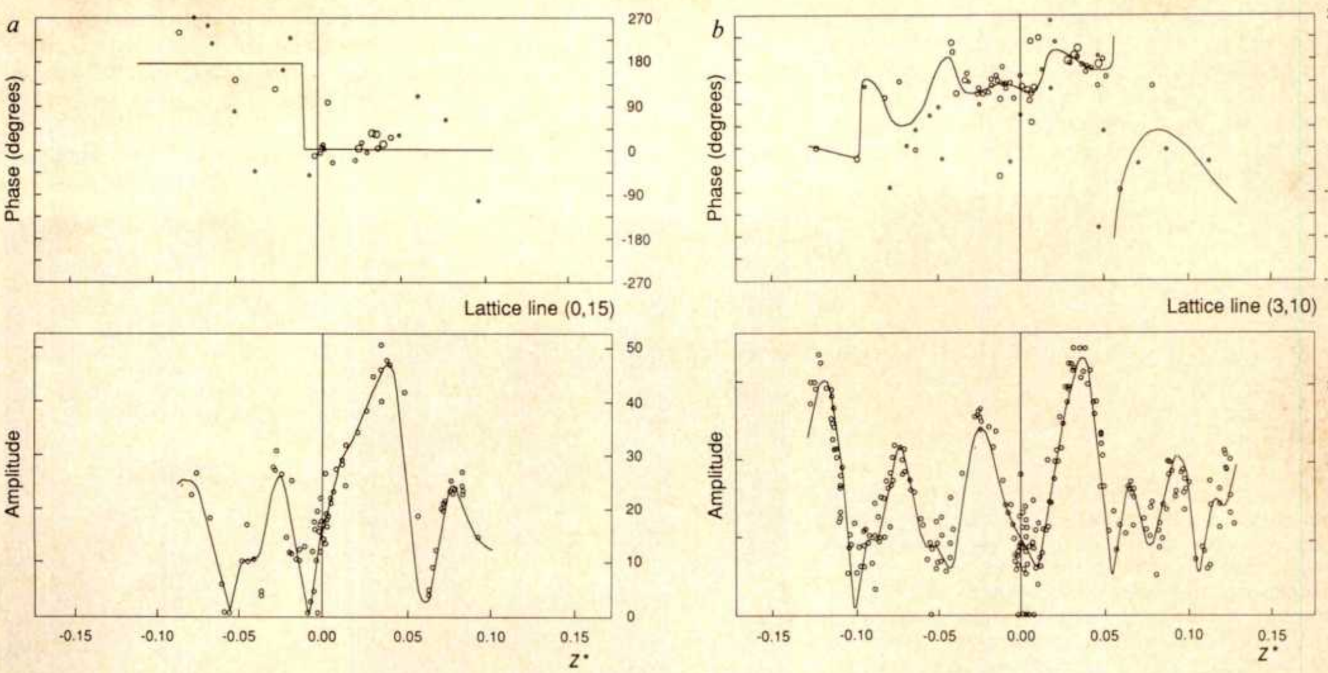


FIG. 1 Variation of phases and amplitudes of structure factors along two out of 115 lattice lines in the asymmetric unit, to 6 Å resolution. The size of circles in the upper half relates to the accuracy of phase measurement (see ref. 8), with larger circles indicating higher accuracy. Due to crystal symmetry, lattice lines with indices (0, k) have real phases of 0° or 180°. *a*, Lattice line (0, 15); 7.33 Å resolution at $z^*=0$. *b*, Lattice line (3, 10); 9.33 Å resolution at $z^*=0$.

METHODS. Two-dimensional crystals measuring up to 10 μm in diameter were grown from LHC-II isolated from pea leaves and specimens for electron crystallography were prepared in the presence of tannin[5]. Electron diffraction patterns yielded structure factor amplitudes to 3.2 Å resolution in all directions except for a 30° missing cone (D.N.W. and W.K., manuscript submitted). Low-resolution amplitudes to 30 Å were taken from image transforms. High-resolution images of tilted and untilted two-dimensional crystals were recorded on Agfa Scientia film in the JEOL 100B cryo-electron microscope equipped with a field emission gun[31] at Berkeley, California, in spotscan mode[32-34] at a specimen temperature of −113 °C, with an electron dose of 5 to 8 e $Å^{-2}$ and magnification ×57,000. Films were developed for 12 min in full-strength Kodak D-19 developer. Areas with high crystallinity selected by optical diffraction were digitized in 6,000 × 6,000 7-μm steps on a Perkin-Elmer 1010 GM microdensitometer. In total, 20 out of about 1,000 images were processed, including four each recorded at tilt angles of 0°, 20° and 30°, six at 45° and two at 60°. The highest actual tilt angle was 57.2°. Image transforms were corrected for defocus gradient, lattice distortions, astigmatism and beam tilt[8,9]. Phases of structure factors were combined, minimizing the residual phase error for each new image. Phase residuals for individual images ranged from 22° to 33° for all reflections above background. Curves were fitted[35] to amplitudes and phases for each lattice line and sampled at $(140\ Å)^{-1}$ to generate a three-dimensional set of structure factors which was 84% complete. The actual resolution in *z* direction was estimated at about 8 Å from the point spread function for the 30° missing cone[36].

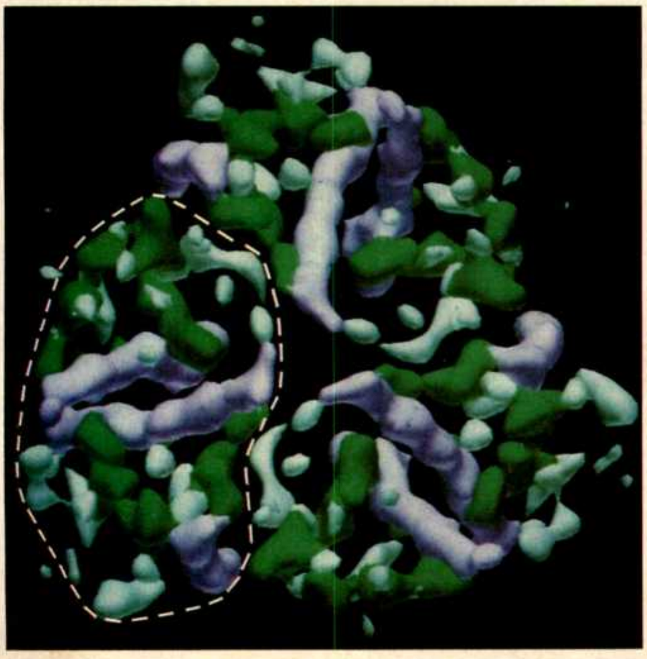
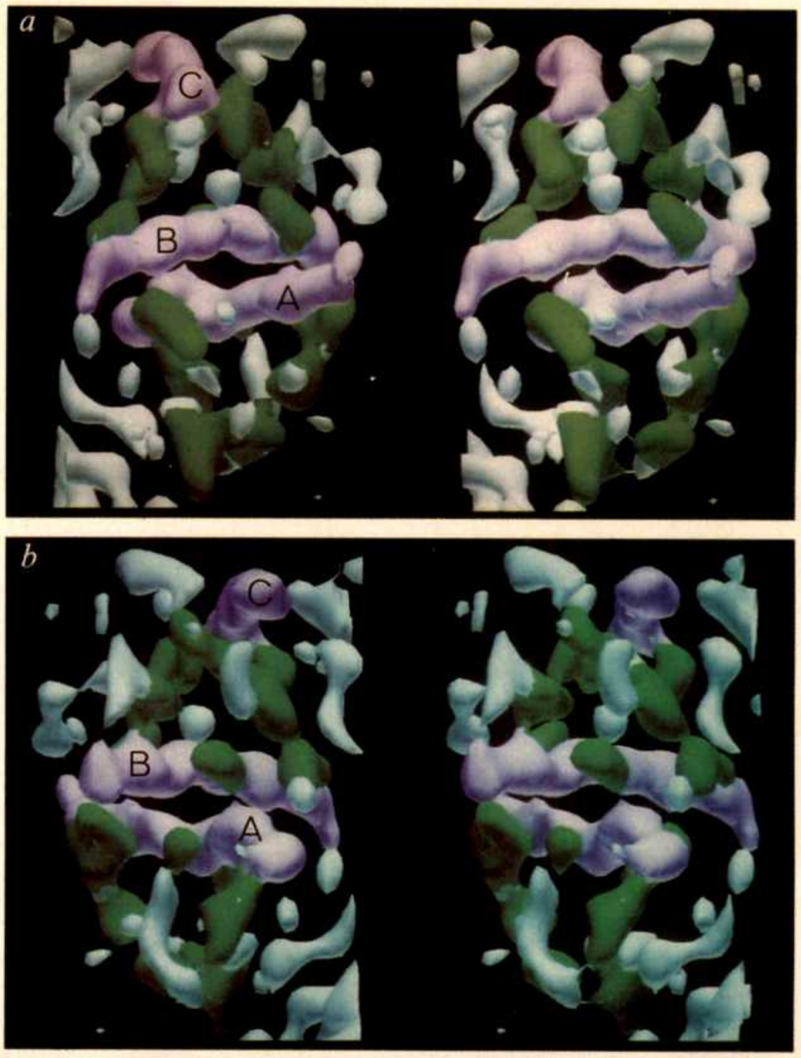


FIG. 2 Three-dimensional map of the LHC-II trimer at 6 Å resolution seen from a position above the membrane plane. Well resolved rod-like features (purple) represent α helices. Small, roughly ellipsoidal centres of density (green) were assigned to porphyrin head groups of chlorophyll molecules. Partly resolved regions of the polypeptide at the membrane surface are white. The trimer consists of three apparently identical monomers, related by crystallographic threefold symmetry. Featureless regions in the centre and around the perimeter of the trimer probably contain disordered lipid and detergent. Solvent flattening[37] reduced the phase error only by about 10°, reflecting the high quality of phases. The map was generated without solvent flattening by standard crystallographic procedures and coloured using a program written by S. Fuller.

of the homologous domains in the sequence suggest that they are on the same surface as the N terminus[14,15] which is thought to be located on the outer, stromal surface[18]. By implication, the map in Figs 2 and 3*a* is viewed from the stromal side. We therefore propose a topography of the polypeptide as shown in Fig. 4, even though we cannot trace the amino-acid chain with certainty at this resolution.

LHC-II, a eukaryotic membrane protein, bears no resemblance to the three prokaryotic membrane proteins, the structures of which have been determined at high resolution: bacteriorhodopsin[9], the bacterial reaction centre[11], and matrix porin from *Rhodobacter capsulatus*[19]. However, LHC-I, the eukaryotic antenna complex closely associated with photosystem I in chloroplasts, should resemble LHC-II structurally. The



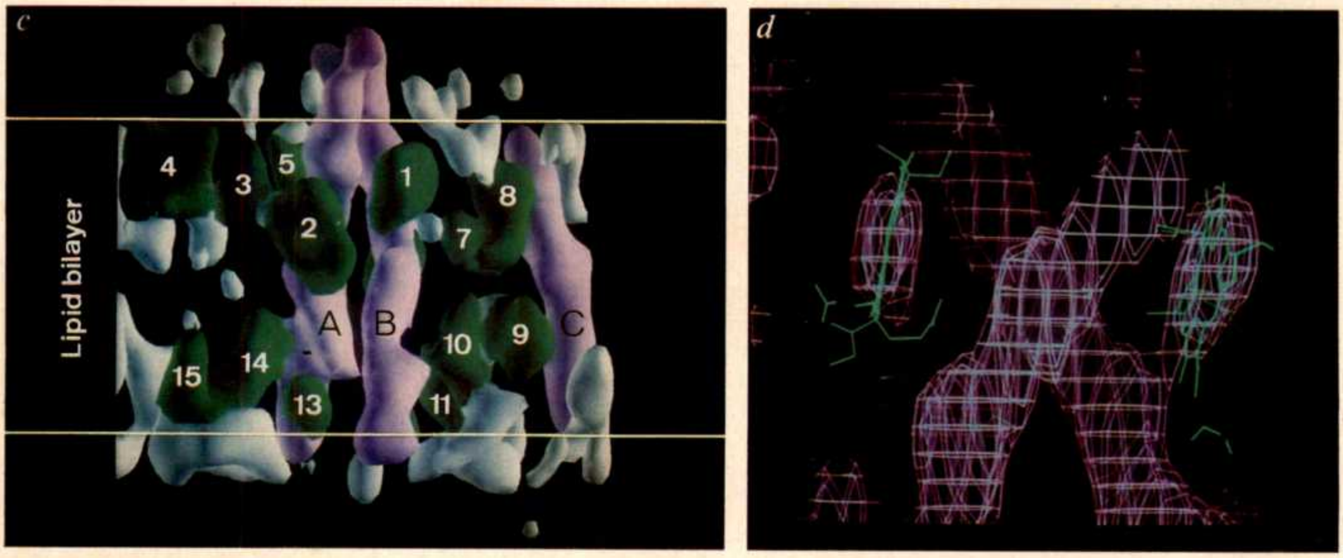


FIG. 3 Views of the LHC-II monomer. Colouring of α helices and chlorophyll porphyrins in *a* to *c* as in Fig. 2. Polypeptide at the membrane surface and density in the membrane belonging to the adjacent monomer is white. Two stereo pairs show the complex as seen from above (*a*) and below (*b*) the membrane. The side view (*c*) is from a central position in the lipid bilayer outside the trimer. Well resolved features are largely confined to the level of the hydrophobic part of the bilayer. The monomer contains three membrane-spanning α helices, labelled A, B and C, plus 15 chlorophyll molecules, labelled 1 to 15 in *c* (chlorophylls 6 and 12 are hidden behind helix B). Helices A and B are inclined by about 30° with respect to the membrane normal. Helix C runs approximately perpendicular to the membrane plane. The upper parts of helices A and B and the four chlorophylls nearby (numbers 1 and 5, 2 and 6) are related by local, twofold symmetry (*a*). This symmetry is not evident on the reverse side of the complex (*b*). Helices A and B seem to protrude at the upper membrane surface (*c*). Helix C appears to be fully embedded in the hydrophobic lipid bilayer. Chlorophyll porphyrins are arranged on two levels, corresponding roughly to the two bilayer leaflets. Some of the chlorophylls have protrusions which may be part of the phytyl chains. *d*, Detail of helices A and B and of chlorophylls 1 and 5, related by the non-crystallographic diad. A molecular model of chlorophyll *a* porphyrin was adapted from bacteriochlorophyll *a* coordinates[20] and fitted to 15 centres of density in the map with similar dimensions.

polypeptide sequence of both complexes indicates significant sequence identity[16], the most highly conserved regions being those which are related by internal homology in LHC-II.

Arrangement of chlorophylls

Another striking feature of the map is the presence of several small, distinct regions of density (green in Figs 2 and 3), surrounding the central pair of α helices in the hydrophobic interior of the complex. These are clearly not part of the polypeptide since this is forced into an α-helical or β-sheet conformation by the hydrophobic environment. Both types of secondary structure can be distinguished easily as such at 6 Å resolution. Most of these centres of density have an ellipsoidal, somewhat angular shape, measuring about 8 Å in diameter and about 4 Å in thickness which leaves no doubt that they represent the planar porphyrin ring systems of chlorophyll molecules. We assigned chlorophylls to 15 such centres of density, in agreement with biochemical findings which indicated a chlorophyll : polypeptide molar ratio of 15:1 (ref. 1). As expected, chlorophyll *a* and *b* cannot be distinguished at this resolution. Figure 3*d* shows how chlorophyll porphyrin rings might fit into two of these regions. All assigned chlorophyll densities were roughly of this size except number 13 which was smaller and may prove not to be a porphyrin at higher resolution. A feature extending into the hydrophobic bilayer near the bottom of helix C has the correct shape and size for a porphyrin but seems too distant from any resolved polypeptide. Otherwise, only few small islands of density are visible in the interior of the complex. These may belong to the linear hydrocarbons of phytyl chains or carotenoids which are not resolved.

The side view (Fig. 3*c*) reveals that the chlorophyll head groups are arranged on two levels, corresponding roughly to the upper and lower leaflet of the lipid bilayer. The upper level (Figs 3*a* and 5*a*) contains eight chlorophylls (numbers 1 to 8), at least four of which (numbers 1 and 5, 2 and 6) are related by the same local twofold symmetry that was found for the upper parts of helices A and B, indicating that there are conserved chlorophyll binding sites in the homologous sequence. Figure 3*d* illustrates the local non-crystallographic symmetry of helices A and B and of chlorophylls 1 and 5. The seven lower chlorophylls (numbers 9 to 15), like the polypeptide on this side of the complex, are apparently not related by twofold symmetry (Figs 3*b* and 5*b*).

Chlorophyll porphyrins are oriented roughly perpendicular to the membrane plane, at an average angle of about 10° from the membrane normal. The centre-to-centre distances between nearest neighbours on each level fall into a narrow range of 9 to 14 Å. Chlorophylls on the upper level are arranged in a ring-like fashion (Fig. 5*a*), so that each has two neighbours. The plane of this ring is inclined to bring chlorophylls 6, 7 and 8 closer to the lower level. The lower chlorophylls describe an approximate crescent shape (Fig. 5*b*) which slopes upwards, with chlorophylls 9, 10 and 11 closest to chlorophylls 6, 7 and 8 on the upper level, at a distance of 13 to 14 Å.

The LHC-II polypeptide contains only three histidines which is the usual ligand for Mg^{2+} of chlorophylls[11,20]. Most chromophores in the complex must therefore be bound by other residues or in other ways. Several chlorophylls seem to be suspended between the polypeptide on the membrane surface and the membrane-spanning α helices. The centres of these chlorophyll porphyrins seem rather too distant from the polypeptide for direct coordination of amino-acid side chains to Mg^{2+}, suggesting that water molecules intervene, or that these chlorophylls are held in place by hydrogen bonding to the perimeter of the porphyrin rings.

Compared with LHC-II, the relationship between chromophores and polypeptide is reversed in the two bacteriochlorophyll-protein complexes of known structure, where pigment molecules are enclosed in a polypeptide cage. The light-harvesting bacteriochlorophyll *a*-protein complex from *Prosthecochloris aestuarii*[20] is a soluble protein, and its polypeptide forms a β barrel to shield the hydrophobic chromophores in the interior. By contrast, the chlorophyll-containing region of LHC-II is surrounded by lipid and need not be shielded. In the bacterial reaction centre[11], the prosthetic groups are partly surrounded by a total of ten α helices creating a special environment which must be responsible for the large red shift of the absorption maximum. In plant photosynthesis, the red shift is less pronounced and therefore such a special protein environment is not required in LHC-II. The role of the polypeptide, and in particular of the tilted helices A and B, seems to be primarily that of a scaffold for packing the chromophores into a small volume, ensuring optimal spacing and orientation for energy transfer.

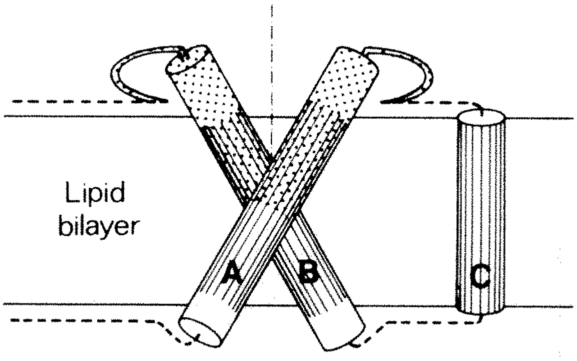


FIG. 4 Proposed topography of the LHC-II polypeptide in the photosynthetic membrane. Hydrophobic regions of helices A, B and C are striped. Shaded regions of helices A and B and their hook-shaped extensions at the top of the complex are related by a non-crystallographic twofold axis running roughly perpendicular to the membrane plane (dash-dotted line) and by internal polypeptide homology. Dashed lines represent domains of the polypeptide on the membrane surfaces that are less well resolved in the map. The order of the three helices in the polypeptide sequence seems to be B, C and A, beginning at the N terminus. The homologous regions comprise the upper half to two thirds of helices A and B and their hook-like extensions. These regions are on the same side as the N terminus[14,15] which is thought to be located on the outer, stromal side of the membrane[18].

The trimer

Trimer formation appears to be mediated mainly by interaction between monomers at the membrane surface, notably at the upper ends of helices B which are less than 7 Å apart, because there are few polypeptide contacts in the hydrophobic interior. A region of low density was chosen as the molecular boundary (dashed line in Fig. 2), so that within one monomer, the same polypeptide chain was closest to all chlorophylls. All other possible boundaries place several chlorophylls close to polypeptides of two different monomers. A boundary between helices A and B could be proposed on the basis that other membrane proteins interact through contacts between hydrophobic α helices[9,11]. Gene duplication, which probably gave rise to the local twofold axis in LHC-II, tends to result in intramolecular rather than intermolecular symmetry, and therefore such a boundary appears unlikely. Nevertheless, the molecular boundary shown here should be regarded as preliminary.

There are several reasons to believe that the trimer is the native, functional conformation of LHC-II. Analytical ultracentrifugation has shown that the trimeric complex is highly stable in detergent solution[1]. Fluorescence kinetics of isolated LHC-II indicated that rapid energy transfer from chlorophylls *b* to *a*, which is characteristic for the functional complex, breaks down as the trimer dissociates[21]. Freeze-fracture electron microscopy of native thylakoid grana revealed a large number of 80 Å particles[22] of the same shape and size as on fracture faces of two-dimensional crystals of LHC-II (ref. 23). Finally, most other known light-harvesting complexes have threefold symmetry[20,24-26]. Energy transfer from an antenna of this sym-

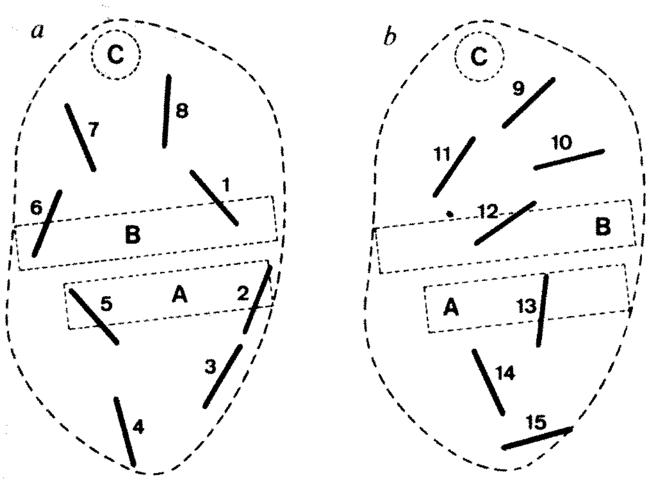


FIG. 5 Approximate position of chlorophylls on the upper level (*a*) and on the lower level (*b*) in the complex. Chlorophyll porphyrins, represented by black bars, are oriented roughly perpendicular to the membrane plane. The outlines of the monomer and of helices A, B and C are dashed. Note that *a* and *b* are seen from above the membrane and thus *b* appears mirror-symmetric to Fig. 3*b*. Chlorophyll 14 is attached to helix A, chlorophyll 10 to helix B and chlorophylls 7 and 8 to helix C. Two chlorophylls (numbers 10 and 15) are only partly resolved from the non-helical polypeptide at the membrane surface. A preliminary estimate of energy transfer rates between chlorophylls was obtained from expressions[29] for the interaction energy and energy transfer times for the two cases of delocalized exciton coupling and Förster transfer, assuming that all chlorophylls were chlorophyll *a*. The dipole strength of chlorophyll *a* was taken as 16.8 Debye (ref. 38), the natural fluorescence decay time as 1.5×10^{-8} s (ref. 39) and the critical distance for Förster transfer at orientation factor 1 as 90 Å (ref. 29). Because the orientation of porphyrin rings cannot be determined with certainty at present, the factor for random orientation, 0.66 (ref. 29), was used in the calculation.

metry to other molecules in the same plane is less dependent on the rotational alignment of the molecules. We therefore conclude that Fig. 2 shows the structure of the complex as it exists in the photosynthetic membrane.

The chlorophyll concentration in the whole volume of the LHC-II trimer is 0.3 M. The chromophore concentration in the trimer of the soluble, bacterial complex from *P. aestuarii*[20] is 0.11 M, even though the local concentration in the central hydrophobic core (0.94 M) is slightly higher than in the corresponding region of LHC-II (0.7 M). Moreover, LHC-II uses only 15 amino-acid residues per bound chlorophyll, whereas the soluble antenna complex uses 50. Overall, the plant light-harvesting complex is thus about three times more effective at organizing chlorophylls and, hence, as a molecular antenna of solar energy.

Energy transfer

In photosynthesis, energy is transferred between chlorophyll molecules mainly by two mechanisms, delocalized exciton coupling and Förster transfer[27]. Exciton coupling occurs between similar or identical chromophores at distances up to 20 Å, depending on the angle between them. Energy oscillates between donor and acceptor without being localized on either molecule. At distances greater than 20 Å, Förster transfer[28] is more favourable. This is a longer-range dipole-dipole interaction through space whereby the acceptor molecule is excited as the donor molecule drops to the ground state.

Based on the observed chlorophyll positions we estimated the rate of energy transfer between chlorophylls in the monomer and in the trimer, assuming that all chlorophylls were chlorophyll *a*. Our calculation indicated that delocalized exciton coupling occurs between chlorophylls in one LHC-II monomer within about 10^{-13} to 10^{-14} s, even when taking the orientation of Qy transition dipole moments as random. Optimal orientation would result in a transfer rate that is about 2 to 3 times faster. Since every chlorophyll can absorb, on average, only one photon per second even in strong sunlight[29], delocalized exciton-coupling seems essential for efficient light-harvesting. Energy transfer between monomers in the same trimer is more likely to proceed by the Förster mechanism because of the greater distance of chromophores.

Owing to interspersed lipid, the average shortest distance between chlorophylls in adjacent trimers in the thylakoid membrane is probably much longer than 10-15 Å, the minimum value assuming van der Waals' contact. This means that Förster transfer is the likely mechanism of energy transmission over long distances in the membrane. The orientation of chlorophylls 2, 3 and 15 at the outer hydrophobic perimeter of the trimer may be favourable for energy transmission to neighbouring light-harvesting complexes or reaction centres.

The site of primary charge separation, to which energy must ultimately be transferred, is the special pair of chlorophylls in the photosystem II reaction centre complex. By homology with the bacterial reaction centre[30], it is located on the inner, lumenal side of the membrane. If the inferred orientation of LHC-II in the photosynthetic membrane is correct, the special pair is on the same side of the membrane as the seven lower chlorophylls. ☐

Received 28 December 1990; accepted 15 February 1991.

1. Butler, P. J. G. & Kühlbrandt, W. *Proc. natn. Acad. Sci. U.S.A.* **85,** 3797-3801 (1988).
2. Kühlbrandt, W. *Nature* **307,** 478-480 (1984).
3. Cashmore, A. R. *Proc. natn. Acad. Sci. U.S.A.* **81,** 2960-2964 (1984).
4. Siefermann-Harms, D. *Biochim. biophys. Acta* **811,** 325-355 (1985).
5. Wang, D. N. & Kühlbrandt, W. *J. molec. Biol.*, in the press (1991).
6. Henderson, R. & Unwin, P. N. T. *Nature* **257,** 28-32 (1975).
7. Baldwin, J. M. & Henderson, R. *Ultramicroscopy* **14,** 319-336 (1984).
8. Henderson, R., Baldwin, J. M., Downing, K. H., Lepault, J. & Zemlin, F. *Ultramicroscopy* **19,** 147-178 (1986).
9. Henderson, R. *et al. J. molec. Biol.* **213,** 899-929 (1990).
10. Chothia, C. *A. Rev. Biochem.* **53,** 537-572 (1985).
11. Deisenhofer, J., Epp, O., Miki, K., Huber, R. & Michel, H. *Nature* **318,** 618-624 (1985).
12. Deisenhofer, J. & Michel, H. *Science* **245,** 1463-1473 (1989).
13. Yeates, T. O., Komyia, H., Rees, D. C., Allen, J. P. & Feher, G. *Proc. natn. Acad. Sci. U.S.A.* **84,** 6438-6442 (1987).
14. Karlin-Neumann, G. A., Kohorn, B. D., Thornber, J. P. & Tobin, E. M. *J. molec. Appl. Gen.* **3,** 45-61 (1985).
15. Bürgi, R., Suter, F. & Zuber, H. *Biochim. biophys. Acta* **890,** 346-351 (1987).
16. Hoffmann, N. E. *et al. Proc. natn. Acad. Sci. U.S.A.* **84,** 8844-8848 (1986).
17. Kühlbrandt, W. & Downing, K. H. *J. molec. Biol.* **207,** 823-828 (1989).
18. Mullett, J. E. *J. biol. Chem.* **258,** 9941-9948 (1983).
19. Weiss, M. S., Wacker, T., Weckesser, J., Welte, W. & Schulz, G. E. *FEBS Lett.* **267,** 268-272 (1990).
20. Matthews, B. W., Fenna, R. E., Bolognesi, M. C., Schmid, M. F. & Olson, J. M. *J. molec. Biol.* **131,** 259-285 (1979).
21. Ide, J. P., Klug, D., Kühlbrandt, W., Giorgi, L. B. & Porter, G. *Biochim. biophys. Acta* **893,** 349-364 (1987).
22. Simpson, D. J. *Carlsberg Res. Comm.* **44,** 305-336 (1979).
23. Kühlbrandt, W., Thaler, T. & Wehrli, E. *J. Cell Biol.* **96,** 1414-1424 (1983).
24. Schirmer, T., Bode, W., Huber, R., Sidler, W. & Zuber, H. *J. molec. Biol.* **184,** 257-277 (1985).
25. Stark, W., Kühlbrandt, W., Wildhaber, I., Wehrli, E. & Mühlethaler, K. *EMBO J.* **3,** 777-783 (1984).
26. Ford, R. C., Hefti, A. & Engel, A. *EMBO J.* **9,** 3067-3075 (1990).
27. Sauer, K. in *Encyclopaedia of Plant Physiology* Vol 19: Photosynthesis III (eds Staehelin, L. A. & Arntzen, C. J.) 85-97 (Springer, Berlin, 1986).
28. Förster, Th. in *Modern Quantum Chemistry* Part 3 (ed. Sinanoglu, O.) 93-137 (Academic Press, London, 1965).
29. van Grondelle, R. *Biochim. biophys. Acta* **811,** 147-195 (1985).
30. Michel, H. & Deisenhofer, J. in *Encyclopaedia of Plant Physiology* Vol 19: Photosynthesis III (eds. Staehelin, A. C., Arntzen, C. J.) 371-387 (Springer, Berlin, 1986).
31. Hayward, S. B. & Glaeser, R. M. *Ultramicroscopy* **5,** 3-8 (1980).
32. Henderson, R. & Glaeser, R. M. *Ultramicroscopy* **16,** 139-150 (1985).
33. Downing, K. & Glaeser, R. M. *Ultramicroscopy* **20,** 269-278 (1986).
34. Bullough, P. & Henderson, R. *Ultramicroscopy* **21,** 223-230 (1987).
35. Agard, D. A. *J. molec. Biol.* **167,** 849-852 (1983).
36. Glaeser, R. M., Tong, L. & Kim, S.-H. *Ultramicroscopy* **27,** 307-318 (1989).
37. Wang, B. C. *Meth. Enzym.* **115,** 90-112 (1985).
38. Shipman, L. L., Cotton, T. M., Norris, J. R. & Katz, J. J. *J. Am. chem. Soc.* **98,** 8222-8230 (1976).
39. Brody, S. S. *Rev. scient. Instrum.* **28,** 1021-1026 (1957).

ACKNOWLEDGEMENTS. We thank R. Henderson for image processing programs, advice and encouragement, K. Dörr for preparing the complex and A. Lahm for advice on crystallographic programs. Special thanks go to K. H. Downing for the use of the cryo-electron microscope at the Lawrence Berkeley Laboratory. Financial support from the Deutsche Forschungsgemeinschaft is gratefully acknowledged.

Iron distribution in the intracluster gas of the Virgo cluster of galaxies

Katsuji Koyama, Shiro Takano & Yuzuru Tawara

Department of Astrophysics, School of Science,
Nagoya University Furo-cho, Chikusa-ku, Nagoya 464-01, Japan

DIFFUSE X-ray emission, particularly fluorescence from the iron K line, from hot gas in the clusters of galaxies provides valuable information on the structure and evolution of the clusters[1]. Because heavy elements such as iron are made in stars, the presence of iron distinguishes gas that originated in galaxies from primordial gas that has remained unprocessed since formation of the cluster. Previous observations of about 20 clusters indicate that iron abundance in the hot gas is about half the cosmic value[2], but these observations were made with non-imaging devices which gave no information on the spatial distribution of the iron. Lack of spatial distribution can also skew the estimated mean iron abundance determined in this way. Here we report evidence of an iron abundance gradient in hot gas in the Virgo cluster of galaxies. Iron is centrally concentrated, indicating that gas at the cluster core has been enriched by galactic mass loss.

The Virgo cluster is the nearest rich cluster of galaxies and has a large optical extension of ~12° along the major axis of the cluster. It is classified as an irregular cluster in which the distribution of the galaxies is unrelaxed. The galaxy distribution has two major concentrations of galaxies around M87 and M49 (ref. 3). We have conducted X-ray scanning observations from south to north along the major axis, which runs over M49 and M87, using the Large Area Counter (LAC)[4] onboard the Ginga satellite[5]. The beam size of the LAC is ~1° and 2° (full width at half maximum, FWHM) along and perpendicular to the scan path, respectively. The LAC is sensitive in the energy band 1–37 keV with a maximum effective area of 4,000 cm^2. With the high sensitivity of the LAC, we found large-scale extended X-ray emission of ~12° covering the whole Virgo cluster[6].

Figure 1*a*, *b* shows typical X-ray spectra summed for the data from the central region (within position angle 0.5° of M87) and outer region (2–3.5° north of M87) of the Virgo cluster, respectively. The X-ray spectrum near the central region cannot be fitted by a single-temperature model but can be fitted by a two-component model of thermal bremsstrahlung (and iron line) and power-law emission. The best-fit photon index and temperature are 1.90 ± 0.15 and 2.19 ± 0.04 keV, respectively (hereafter, we quote errors at the 90% confidence level of the parameter of interest). The best-fit models are given by the histograms in Fig. 1*a*.

To separate the power-law component from the optically thin thermal intracluster emission, we obtained X-ray spectra at each position of the scan path. To these spectra, we fitted the two-component model, fixing the photon index at 1.9. The free parameters of these fits are the normalization factor of the power-law component, the temperature and normalization factor of the thermal-bremsstrahlung component, and the central energy and equivalent width of iron line. The fits were generally acceptable, with the reduced χ^2 ranging from 0.6 to 1.3. The best-fit normalization factor of the power-law component becomes negligibly small when the scan angle is more than ~1° from M87. In this region we tried fits to a single-temperature thermal-bremsstrahlung model and found the fits to be acceptable with the reduced χ^2 ranging from 0.8 to 1.2. The histogram of Fig. 1*b* is an example of the best fit to a single-temperature thermal-bremsstrahlung model with temperature of 2.05 ± 0.16 keV.

Using additional scans near the cluster centre, we found that the power-law emission is due to the active galactic nuclei of M87 and Seyfert 2 galaxy NGC4388 (ref. 7). Hanson *et al.*[9] also found no hard-X-ray emission other than that from M87 and NGC4388 within ~4° of M87. We therefore conclude that the power-law component is due only to M87 and NGC4388.

Figure 2 shows the best-fit temperature distribution along the scan path. In the region within ~1° of M87, the best-fit temperature is the result of two-component fit, whereas that of the region farther than ~1° from M87 is the best-fit temperature of a single-component model. We find that the temperature of intracluster gas is not isothermal but shows significant increase from 2 keV to 3.6 keV toward the south part of the cluster.

At most scan positions, we found significant iron-line emission at ~6.7 keV, a value consistent with that expected from plasma at a temperature of 2.0–3.6 keV in ionization equilibrium. The active galactic nuclei M87 and NGC4388 can also be iron-line emitters. The iron equivalent width of a Seyfert 2 galaxy is generally larger than that of the other class of active galactic nuclei[8] but the hydrogen column density of NGC4388 is $\sim2\times10^{23}$ H cm^2 (ref. 9). In this range the equivalent width is about 200–300 eV (ref. 8). We made a rather conservative estimate of the possible contamination by the iron-line flux from M87 and NGC4388, assuming that the iron-line equivalent widths are ~300 eV, and found that the contamination from these active galactic nuclei is only 10% of the total iron-line flux. We therefore neglect contamination by the iron-line flux from these active galactic nuclei in the following discussion.

We derived the equivalent width of the iron line by dividing the best-fit iron flux by the flux of thermal-bremsstrahlung component at 6.7 keV. The continuum emission of the power-law component due to M87 and NGC4388 has therefore already been removed. The equivalent width can be converted to iron abundance using the calculated equivalent width of the cosmic abundance under the assumption of ionization equilibrium. The iron abundance distribution is plotted in Fig. 3 as a function of

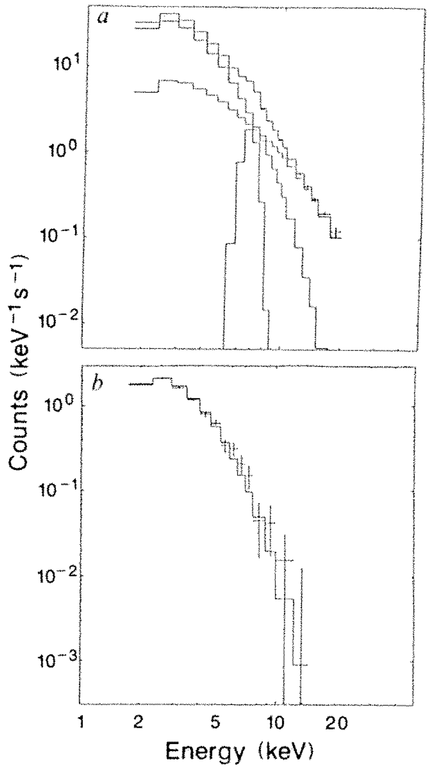


FIG. 1 X-ray spectra summed for the scan data *a*, within the position angle 0.5° from M87 and *b*, 2–3.5° north from M87. The solid histograms are from the best-fit model described in the text.

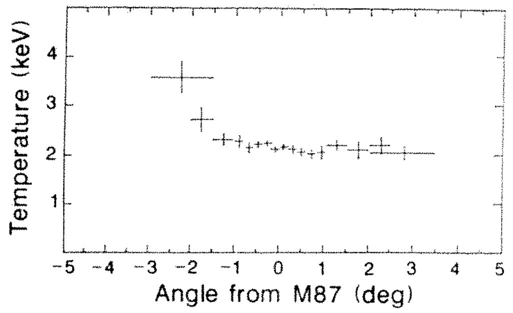


FIG. 2 The best-fit temperature distribution along the scan path from south (negative angles) to north (positive angles). In the region within ~1° of M87 the results are those of a two-component fit, and those in the outer region are the best-fit parameters of a single-temperature model (see text). The scan profile is not deconvolved from the collimator response (1° FWHM).

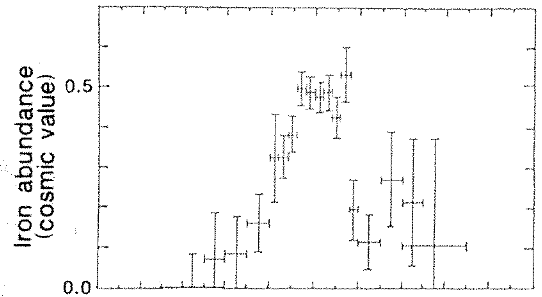


FIG. 3 Iron abundance as a function of position angle from M87, where the scan profile is not deconvolved from the collimator response (1° FWHM).

scan angle. The calculated equivalent width of the cosmic abundance is in the range 1.4–1.7 keV in the relevant temperature range of 2.0–3.6 keV (ref. 10). Therefore any systematic error due to a temperature gradient is at most ~10%.

The distribution of iron abundance shows a clear peak near the cluster centre at M87. The iron abundance in the outer region of the cluster (more than 1° from M87) is about 0.1–0.2 of the cosmic value. Because the angular profile of the iron abundance is comparable to that of the collimator response (1° FWHM), we cannot resolve the detailed structure of the iron abundance within 1° of M87, but we can safely conclude that the mean iron abundance in the central region (within 1° of M87) is ~0.5 of the cosmic value. The previous result[11] of mean abundance is roughly consistent with our result for the central region. This is reasonable because the mean abundance is a luminosity-weighted mean. Because luminosity is a function of the square of gas density, the cluster centre with high gas density gives an over-estimation of the mean abundance. The gas-density-weighted mean would give a more realistic abundance. From the two-dimensional mapping of the cluster gas, we estimate that the total mass of gas in the Virgo cluster is about 10 times larger than that of the central region (within 50′ of M87)[12]. Therefore the 'true' mean abundance of the Virgo cluster should be similar to that of the outer region (0.1–0.2 of the cosmic value). As the mean abundance of the Virgo cluster is only 0.1–0.2 of the cosmic value, we conclude that the intracluster gas of Virgo is not mainly due to matter outgassed from the member galaxies. By contrast, the central region of the cluster has been contaminated by more efficient mass loss from galaxies because the galaxy distribution is denser than that in the outer region of the cluster. We note that Ponman *et al.*[13] reported a similar central concentration of iron abundance from the Perseus cluster of galaxies. Our results for the Virgo cluster, as well as those for the Perseus cluster, clearly demonstrate that spatially resolved iron-line spectroscopy is necessary to investigate the origin and evolution of the intracluster gas. ☐

Received 23 July 1990; accepted 17 January 1991.

1. Sarazin, C. L. *Rev. mode. Phys.* **58,** 1–116 (1986).
2. Rothenflug, R. & Arnaud, M. *Astr. Astrophys.* **144,** 431–442 (1985).
3. Binggeli, B., Tamman, G. A. & Sandage, A. *Astr. J.* **94,** 251–277 (1987).
4. Turner, M. J. L. *et al. Publs astr. Soc. Japan* **41,** 345–372 (1989).
5. Makino, F. *et al. Astr. Lett. Commun.* **25,** 223–233 (1987).
6. Takano, S. *et al. Nature* **340,** 289–290 (1989).
7. Takano, S. & Koyama, K. *Publs astr. Soc. Japan* (in the press).
8. Awaki, H. *et al. Publs astr. Soc. Japan* (in the press).
9. Hanson, C. G. *et al. Astr. Astrophys.* **242,** 262–266 (1990).
10. Mewe, R. *et al. Astr. Astrophys. Suppl.* **65,** 511 (1986).
11. Serlemitsos, P. J. *et al. Astrophys. J.* **211,** L63–L67 (1977).
12. Fabricant, D., Lecar, M. & Gorenstein, P. *Astrophys. J.* **241,** 552–560 (1980).
13. Ponman, T. J. *et al. Nature* **347,** 450–452 (1990).

ACKNOWLEDGEMENTS. We thank C. Day and M. Lim for comments. This work was supported by the Japanese Ministry of Education, Science and Culture.

Irradiation-driven mass transfer in low-mass X-ray binaries

Ph. Podsiadlowski

Institute of Astronomy, University of Cambridge, Madingley Road, Cambridge CB3 0HA, UK

IN a low-mass X-ray binary (LMXB), mass accreted onto a neutron star from a larger companion generates a flux of X-rays which irradiate the companion. Previous studies of irradiated stars have been restricted to the effects on their outermost layers[1–5], or did not address conditions found in interacting binaries[6]. Here I show that, under the radiative flux typical of a LMXB, the companion will expand towards a new state of thermal equilibrium, and that this expansion provides a new mechanism to drive mass transfer onto the neutron star. The evolution of LMXBs can be drastically altered in this way. In particular, this mechanism may introduce a new evolutionary phase during which the orbital period increases, leading to larger orbital periods during and at the end of mass transfer, and (if the companion is a subgiant) shortening the duration of the LMXB phase. These modifications may help to resolve a number of observational puzzles concerning the numbers and properties of LMXBs and related objects.

The main effect of external irradiation is to change the star's effective surface boundary condition, particularly by altering the degree of ionization of hydrogen at the bottom of the irradiated layer (which defines the effective surface boundary). Figure 1 illustrates the effect of irradiation on the radius, R, for various unevolved stars of mass M as a function of external flux, F. In these calculations, the star was assumed to be in thermal equilibrium, and all the external flux was deposited below the photosphere, where the total energy deposited as a function of Thompson optical depth, $\tau_{\rm Th}$, was chosen to be proportional to $(1-e^{-\tau_{\rm Th}})$. Figure 1 shows that, for a given mass ($M \lesssim 1\ M_{\odot}$), there are two flux regimes, separated by a characteristic flux $F_{\rm Ch} \approx 10^{11}$–$10^{12}$ erg s^{-1} cm^{-2}, within which the equilibrium radius, and thus the structure of the star as a whole, changes only slowly with changing external flux. The difference between the two regimes is that, for $F \ll F_{\rm Ch}$, hydrogen at the bottom of the irradiated layer is mainly non-ionized, whereas for $F \gg F_{\rm Ch}$ it is completely ionized (and the star is completely radiative). We find that the overall behaviour of irradiated stars is not very sensitive to how energy is deposited in its outer layer. Indeed, our results would be qualitatively very similar if we had

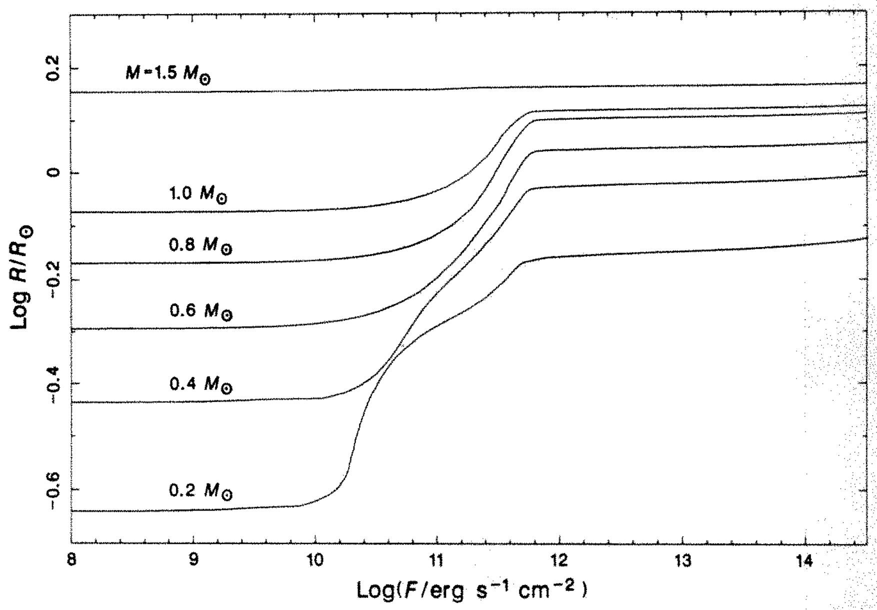


FIG. 1 Equilibrium radius, R, as a function of external irradiation flux, F, for unevolved stars of mass M.

deposited no energy below the photosphere but only changed the surface boundary condition (see ref. 6).

Although these calculations assume that the irradiated star is in thermal equilibrium, this will not be true initially for the irradiated secondaries in LMXBs, because we expect the timescale for the turn-on of the system's X-ray luminosity generally to be short compared with the thermal timescale of the secondary's envelope. This means that, immediately after turn-on of the X-ray flux, the secondary, which 'suddenly' has to adjust to a new surface boundary condition, will be out of thermal equilibrium and will start to relax towards its new equilibrium configuration (shown in Fig. 1). The timescale for this relaxation, $t_{\rm rel}$, is approximately the thermal timescale of the convective envelope, because a radiative core, if it exists, is little affected by a change of the surface boundary conditions; that is, $t_{\rm rel} \approx GM_2\Delta M/2RL$, where M_2 is the total mass of the secondary, ΔM is the mass in its convective envelope, R is its radius, and L is the secondary's internal luminosity. For $M_2 \approx 0.8\ M_\odot$, for example, $t_{\rm rel} \approx 10^7$ yr. It is worth emphasizing that the energy required to inflate the star to its new equilibrium radius is not supplied by the external radiation flux (the star is not heated from the outside), but consists almost entirely of energy generated in the secondary's core. This explains why $t_{\rm rel}$ is independent of the external flux. A detailed description of the relaxation process will be published elsewhere (Ph.P., manuscript in preparation).

We are now in a position to discuss, at least qualitatively, how irradiation effects modify the evolution of LMXBs. In general, we can distinguish four phases: a turn-on phase, a relaxation phase, a quasi-equilibrium phase and a turn-off phase. The turn-on phase will be modified because of a strong positive feedback between the initial expansion and the external irradiation. The most important new feature, however, is the existence of a relaxation phase, in which mass transfer is driven by the thermal relaxation of the secondary's envelope. The secondary expands during this phase, so the orbital period will generally also increase (provided that the secondary can maintain Roche-lobe contact). After the secondary has achieved its new larger equilibrium configuration, mass transfer would stop if there were no other mechanisms to drive it. If there are other driving mechanisms, such as gravitational radiation and/or magnetic braking, mass transfer may continue, but the further evolution will now be governed by these other mechanisms and will be qualitatively similar to the case where irradiation effects are not included (in particular, the orbital period may now decrease; see, for example, ref. 7). The main difference is that the secondary and the orbital period will be larger, because the secondary passes through a different sequence of quasi-equilibrium models (see Fig. 1). Unlike the case without external irradiation, however, mass transfer will stop abruptly when the external irradiation flux falls below a certain critical flux $F_{\rm cr} \sim F_{\rm Ch}$. This occurs as a result of recombination of hydrogen at the bottom of the irradiated layer, whereupon the secondary starts to shrink more rapidly than the orbit can, because of angular momentum losses from the system, and the secondary becomes detached from its Roche lobe.

Unfortunately, the details of the evolution of the LMXB phase are very sensitive to the evolutionary state of the secondary and to the details of the angular-momentum loss mechanisms, and not all of these phases may be realized for any particular set of assumptions. We have calculated the evolution of two typical systems: in case A, the secondary was an unevolved main-sequence star of mass $M_2 = 0.8\ M_\odot$; in case B, it was a subgiant of the same mass. In these calculations, we assumed that the luminosity of the neutron-star primary, which had an initial mass $M_1 = 1.4\ M_\odot$, was equal to its accretion luminosity, where the accretion rate was not allowed to exceed the Eddington accretion rate for a neutron star (if the secondary's mass loss rate exceeded the Eddington rate, we assumed that the excess mass was lost from the system). We also included angular-momentum losses resulting from gravitational radiation, magnetic braking (using the heuristic model of Verbunt and Zwaan[8]), and mass loss from the system.

In Fig. 2 we show the mass-loss rate of the secondary as a function of time after the beginning of the mass-transfer phase. In case A (lower solid curve), our results show many similarities to previous calculations that did not include irradiation effects (see for example ref. 7). The main differences are that the orbital period increases during the first $\sim 10^8$ yr from 5.7 to $\sim$10 h, and that mass transfer stops after $\sim 10^9$ yr, when the secondary's mass has decreased to $\sim 0.15\ M_\odot$, and the period is $\sim$8 h. In case B (upper solid curve), the evolution is altered drastically. The secondary's mass-loss rate always exceeds the neutron star's Eddington accretion rate (dot-dashed curve) by more than an order of magnitude, and lasts for only $\sim 10^6$ yr. When the system becomes detached, the orbital period has increased from 45 h initially to a final period of 90 h, and the mass of the secondary has been reduced to $M_2 \approx 0.2\ M_\odot$. Indeed, because of the

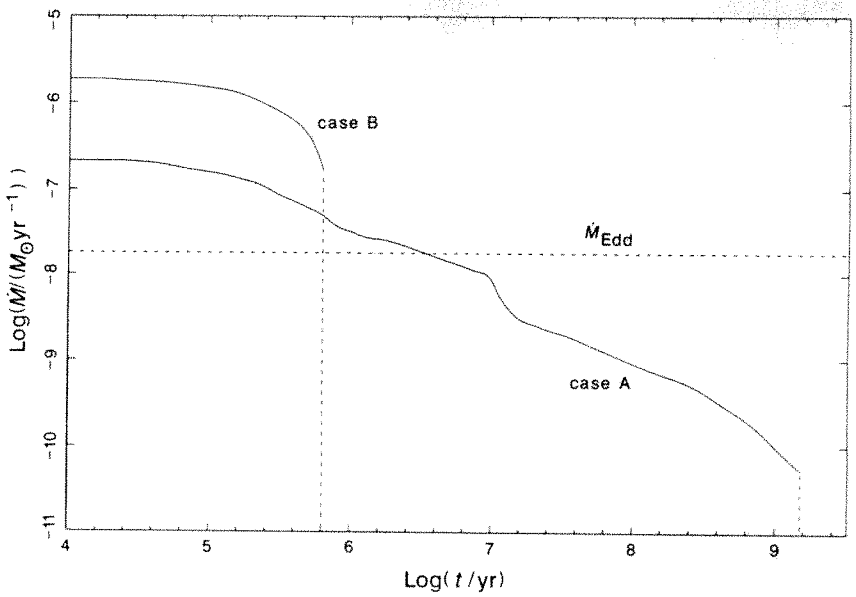


FIG. 2 Mass loss rate, $\dot{M}$, of the secondary in an irradiated LMXB as a function of time, *t*, after the beginning of the mass-transfer phase, for two illustrative binary cases. In case A, the secondary is an unevolved star of mass 0.8 $M_\odot$; in case B, it is a slightly evolved subgiant of the same mass. The initial orbital periods are 5.7 h and 45 h respectively, and the final periods are 8 h and 90 h respectively. In both cases, the secondary is assumed to be a neutron star with an initial mass of 1.4 $M_\odot$, and the calculations include angular-momentum losses resulting from gravitational radiation, magnetic braking and mass loss from the system. For comparison, the dot-dashed curve shows the Eddington accretion rate for a neutron star.

secondary's large mass-loss rate, it may be necessary to include other effects, which may further alter the evolution of LMXBs of this type (as will be discussed elsewhere).

Although these calculations are only exploratory, they illustrate how irradiation effects may explain a variety of observed features of LMXBs that can not easily be understood within the present theoretical framework. First, if the secondary is a main-sequence star, there is a phase in which the secondary expands and the orbital period increases. Indeed, the rate at which the orbital period increases in our model calculation is of the same order as is observed in Cyg X-3[9] and X 1822-371 (ref. 10) ($\dot{P} \approx 0.01$ s yr^{-1}). Thus, these systems may provide direct observational evidence for the existence of a relaxation phase. Second, the radius of the secondary and the orbital period of systems with main-sequence secondaries are significantly larger than in calculations that do not include irradiation effects. As a result, systems such as AC211 (4U2127+11) may have main-sequence instead of subgiant secondaries[11]. (We note that our subgiant calculation seems to be inconsistent with the present characteristics of AC211.) Third, in our calculations mass transfer stops abruptly when the external flux drops below a critical value. This introduces a low-luminosity cutoff in the luminosity distribution of LMXBs (at $\sim 10^{36}$ erg s^{-1} in our model calculation), which is consistent with the observed luminosity distribution[12]. Such a rapid cutoff may also be required to avoid significant spin-down of the neutron-star primary (for a discussion of this point see, for example, ref. 13). Fourth, when the binary becomes detached, the radius of the secondary is larger by a factor of 2–3 than its equilibrium radius without external illumination. This may explain why, in systems such as the binary millisecond pulsar PSR 1957+20, the secondary significantly underfills its Roche lobe[14]. Indeed, in our model calculation with a main-sequence secondary, the system at the end of the mass-transfer phase is very similar to PSR1957+20. Fifth, LMXBs with subgiant secondaries (with periods of less than ~30 days) are expected to radiate near the neutron star's Eddington limit (which may explain the high luminosity of systems such as Sco X-1), and the duration of the LMXB phase may be much shorter than has been found in earlier calculations[15]. If there is a sufficiently large population of LMXBs with subgiant secondaries, this could solve the apparent statistical discrepancy between the number of LMXBs and millisecond pulsars (see, for example, ref. 16).

We expect irradiation effects to be most important for LMXBs, but they may also lead to observational consequences in cataclysmic variables (CVs). As the irradiation fluxes in CVs are much smaller than in LMXBs, and because the energy spectrum is much softer, the effects will generally not be large. Nevertheless, we estimate that the secondaries in CVs may be inflated by up to 10% as a result of external irradiation. The secondary will abruptly become detached from its Roche lobe when the external flux drops below a critical flux. In principle, this could provide an alternative explanation for the period gap in the period distribution of CVs (see, for example, ref. 17).

Finally, we emphasize that the picture outlined here is very preliminary. There are other effects that have not been included but which may change our conclusions quantitatively as well qualitatively. The most important of these are the effects of aspherical illumination, which are likely to lead to internal non-radial circulation; radiation-induced winds[1], which will be particularly important near the end of and after the semi-detached phase; and shielding effects (for example, owing to a flaring disk), which may introduce a negative feedback and may limit the maximum mass-transfer rate.

Despite these caveats, we conclude that, because of the simplicity of the physical process and the large numbers of potential applications, irradiation effects as discussed in this letter are likely to be important in some, if not many, circumstances, and that the theory of the evolution of low-mass X-ray binaries will not be complete without their inclusion. ☐

Received 20 November 1990; accepted 28 January 1991

1. Ruderman, M., Shaham, J., Tavani, M. & Eichler, D. *Astrophys. J.* **343,** 292–312 (1989).
2. Arons, J. *Astrophys. J.* **184,** 539–550 (1973).
3. Bath, G. T. *Mon. Not. R. astr. Soc.* **171,** 311–328 (1975).
4. Osaki, Y. *Astr. Astrophys.* **144,** 369–380 (1985).
5. Hameury, J. M. King, A. R. & Lasota, J. P. *Astr. Astrophys.* **162,** 71–79 (1986).
6. Tout, C. A., Eggleton, P. P., Fabian, A. C. & Pringle, J. E. *Mon. Not. R. astr. Soc.* **238,** 427–438 (1989).
7. Rappaport, S., Verbunt, F. & Joss, P. C. *Astrophys. J.* **275,** 713–731 (1983).
8. Verbunt, F. & Zwaan, C. *Astr. Astrophys.* **100,** L7–L9 (1981).
9. van der Klis, M. & Bonnet-Bidaud, J. M. *Astr. Astrophys.* **214,** 203–208 (1989).
10. Hellier, C., Mason, K. O., Smale, A. P. & Kilkenny, D. *Mon. Not. R. astr. Soc.* **244,** 39p–43p (1990).
11. Naylor, T., Charles, P. A., Drew, J. E. & Hassall, B. J. *Mon. Not. R. astr. Soc.* **233,** 285–304 (1988).
12. Long, K. S. & Van Speybroeck, L. P. in *Accretion-Driven Stellar X-Ray Sources* (ed. Lewin, W. H. G. & Van den Heuvel, E. P. J.) 117–146 (Cambridge University Press, 1983).
13. Ruderman, M., Shaham, J. & Tavani, M. *Astrophys. J.* **336,** 507–518 (1989).
14. Djorgovski, S., & Evans, C. R. *Astrophys. J.* **335,** L61–L65 (1988).
15. Webbink, R. F., Rappaport, S. & Savonije, G. J. *Astrophys. J.* **270,** 678–693 (1983).
16. Ray, A. & Kluźniak, W. *Nature* **344,** 415–417 (1990).
17. Patterson, J. *Astrophys. J. Suppl.* **54,** 443–493 (1984).

Plasma fireballs formed by microwave interference in air

Y. H. Ohtsuki* & H. Ofuruton†

* Department of Physics, Waseda University, Okubo 3, Shinjuku-ku, Tokyo 169, Japan
† Tokyo Metropolitan College of Aeronautical Engineering, 53-1, Minamisenju 8, Arakawa-ku, Tokyo 116, Japan

DESPITE many experimental and theoretical studies, a widely accepted explanation of ball lightning has yet to be found. It is possible, however, that ball lightning is created by the interaction of different types of energy in the atmosphere[1]. Indeed, the production of a fireball by an electric discharge in an atmosphere containing dilute fuel gases such as propane has been reported[2]. We have also managed to produce several types of fireball by setting off electric discharges in an atmosphere containing aerosol with varying concentrations of ethane and/or methane[3]. Here we report the production of plasma fireballs in a natural atmosphere by microwave interference. The fireballs exhibited certain properties that match eyewitness observations of ball lightning, such as motion against the wind and the ability to pass through a wall intact.

Kapitza proposed an alternative theory of ball lightning in which he predicted where fireballs might form[4]. He suggested that the most favourable places for the production of ball lightning were regions where radio waves, produced by thunderstorms, reached their highest intensity—such places correspond to the potential antinodes obtained in various interference phenomena.

Babat[5] used a 1-10-MHz continuous-wave (c.w.) microwave, producing a discharge and hence a fireball at normal atmospheric pressure at distances of several centimetres away from the electrodes. Hamilton[6] and Richie[7] used pulsating microwaves and produced ball-lightning-like discharges at lower pressures. Silberg[8] used a 105-MHz c.w. power oscillator in the air with a Lecher wire and produced a standing-wave electric discharge in the region of an antinode in the electric field along the Lecher wires. Powell and Finkelstein[9] produced plasma fire from the interference of the electromagnetic waves in a glass tube at normal atmospheric pressure, thereby partly confirming Kapitza's model.

But it has proved very difficult to create in the laboratory a plasma fireball that will float and move for a considerable length of time in a natural atmosphere by means of microwave interference.

A schematic diagram of the apparatus used is shown in Fig. 1. We used a 2.45-GHz, 5-kW (maximum power) microwave oscillator (magnetron) operating continuously in the air, and it was usually turned on when a plasma fireball appeared. The microwave radiation was guided through a rectangular waveguide to a cylindrical cavity either made of copper plate or aluminium punched plate. The cavity was 161 mm in diameter and 370 mm in length, and the cross-section of the waveguide was 55 × 109 mm. The waveguide was connected to the cavity in such a way that the short side of the waveguide was parallel to the centre axis of the cavity. One side of the cavity was covered by a piece of immovable aluminium foil and the other side was covered by a movable aluminium punched plate. The air in the cavity was at normal atmospheric pressure. A video camera was set up to record the plasma fireballs as they appeared in the cavity. The lifetime of the plasma fireball (from its appearance to its disappearance) was determined from the video picture.

We observed various kinds of plasma fires at powers ranging from 1 to 5 kW, all, except the last type described below, under the same experimental conditions. The first type of plasma fire observed was stable and practically stationary, but several times was observed to split into two. The fireball lasted for a few minutes (depending on the continuity of the interference in the cavity). Figure 2*a* shows the change in the shape and size of the fireballs observed. Although the electric field was probably very strong just under the waveguide, not all of the fireballs appeared in that area. A standing wave should be set up in the cavity by interference between a propagating wave and a reflected wave, and in this case there should be theoretically six antinodes at which the electric field is strongest. But only one plasma fireball was observed to form at a nodal point, and this corresponded to the point of maximum interference because of a small deviation of the cavity's shape from an ideal cylinder.

The second type of plasma fire produced was a very bright homogeneous flash occupying the whole of the semi-cavity. This flash was white in colour, and very brief (~0.2 s), following which a ball of plasma appeared. This fireball subsequently divided into two, which moved up and down, horizontally and around in the cavity (see Fig. 2*b*). They were probably moving along intense electric field lines in the cavity, their complicated paths therefore the result of the complex electric-field patterns set up in the cavity. These fireballs were orange and lasted for ~2-3 s.

The third type of plasma fire took the form of flames located at the end of the waveguide and separated from the cavity wall (Fig. 2*c*). The colour of the inside of the cavity changed from white and red to blue and orange. The size of the flame varied from 1 to 3 cm, and it lasted for a few seconds only. This type of plasma fire often moved into the waveguide from the cavity. When a 3-mm-thick ceramic board was inserted between the cavity and the waveguide, the plasma fireball moved through the ceramic board intact without causing any damage or melting. This phenomenon is reminiscent of reports of ball lightning moving through a wall or roof.

The fourth type of plasma fire observed was, like the first type, stable. But it flowed up out of the cavity into free space and the whole cavity was illuminated by the discharge. The plasma fireball burnt a hole in the aluminium foil that covered one side of the cavity. A few seconds later, the plasma fire emerged from a hole in the aluminium foil (see Fig. 3). This plasma fire lasted for ~1-2 s, even after the magnetron was turned off, but its occurrence was very rare (usually it was necessary to continue supplying energy to produce fireballs that lasted a considerable length of time). This phenomenon may be the result of the large time constant in the decay of energy stored in the cavity; however, we can find no reasonable explanation for the plasma fire lasting for such a long time after the magnetron has been turned off. The visible plasma flame reaching through the hole was not caused by combustion of the aluminium foil, because the hole did not become any bigger while the plasma flame was visible.

In our final experiment, a copper bar (100 mm in length) was placed just under the waveguide in the cavity. We then observed

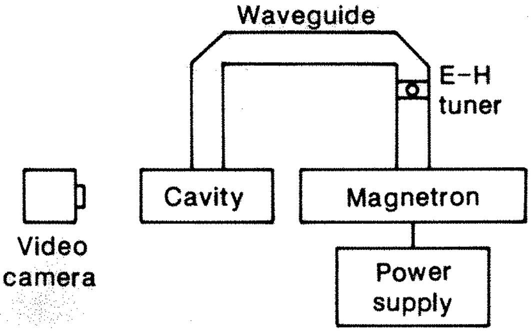


FIG. 1 Schematic diagram of the apparatus. The cavity is 161 mm in diameter and 370 mm in length. The cross-section of the waveguide has dimensions 55 × 109 mm.

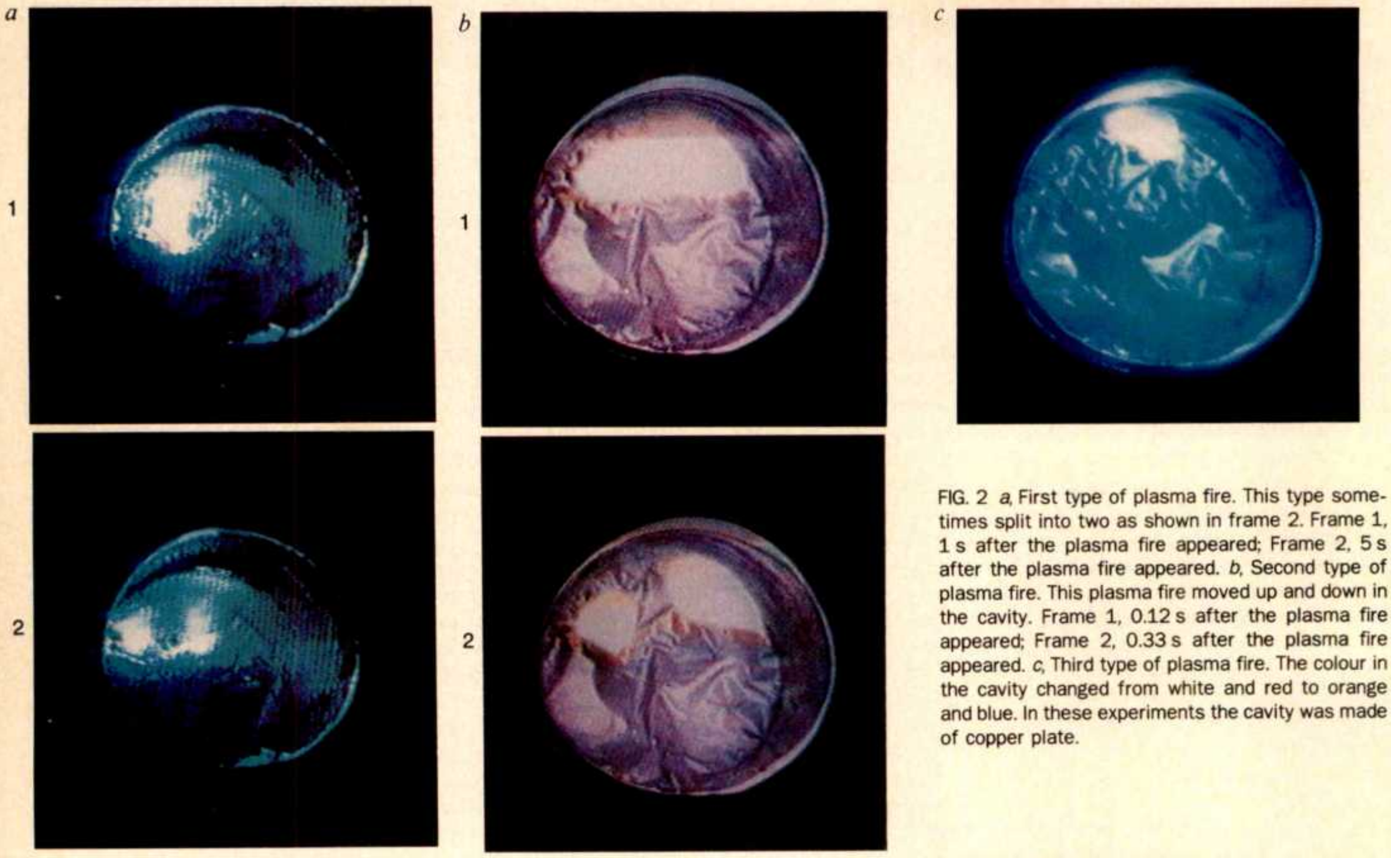


FIG. 2 *a*, First type of plasma fire. This type sometimes split into two as shown in frame 2. Frame 1, 1 s after the plasma fire appeared; Frame 2, 5 s after the plasma fire appeared. *b*, Second type of plasma fire. This plasma fire moved up and down in the cavity. Frame 1, 0.12 s after the plasma fire appeared; Frame 2, 0.33 s after the plasma fire appeared. *c*, Third type of plasma fire. The colour in the cavity changed from white and red to orange and blue. In these experiments the cavity was made of copper plate.

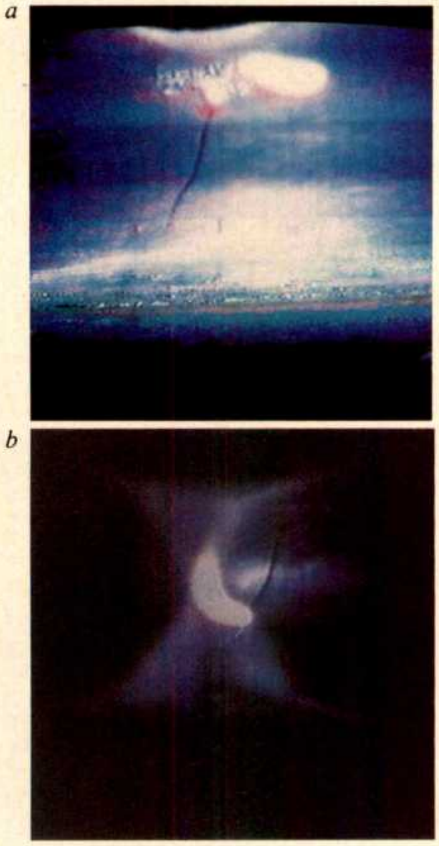


FIG. 4 Fifth type of plasma fire: *a*, 5 s after the plasma fire appeared; *b*, 20 s after the plasma fire appeared. The cavity was made of aluminium punched plate.

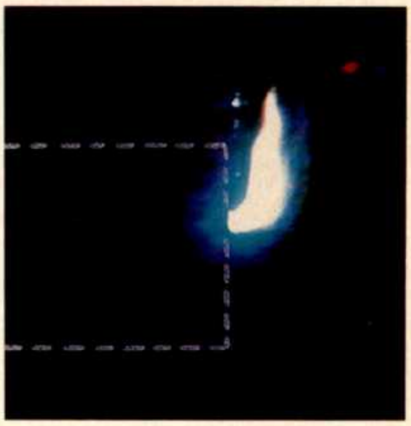


FIG. 3 Fourth type of plasma fire. This type blew out of the cavity. White dashed lines indicate the outline of the cavity (made of aluminium punched plate).

this fifth type of plasma fire at the top of the bar. Many plasma balls appeared one after another, but the lifetime of each was very short (~1 s). Some of them travelled down along the copper bar, and some of them became detached. Interestingly, many witnesses of ball lightning have reported seeing it travel along an electric wire (see Fig. 4). Moreover, when a strong wind from a compressor was blown into the cavity, some plasma balls moved against the wind, indicating that the fireballs will move along an intense electric field even if there is an opposing flow of air. None of the plasma fireballs formed in our experiments was in contact with the cavity wall.

Electromagnetic wave interference is rarely observed in the natural world, and our experiments do not therefore elucidate the process of excitation responsible for natural ball lightning or the energy source for its production. There have, however, been a few studies of the effect of electromagnetic radiation in a natural atmosphere[12,13], and recently Zheng[14] made a calculation of the radiation intensity required to produce a fireball effect. But further theoretical and experimental studies are needed. We believe that electromagnetic interference might be found in the natural atmosphere where the topography is complex and where there is rapid variation in atmospheric electricity. The analogies that we found between the properties of experimental fireballs and those reported for ball lightning seem to confirm the role of such electromagnetic interference.

We are now attempting to use various kinds of semi-cavity, with a varying composition of aerosol, vapour and fuel gases. We also intend to examine Handel's 'caviton model'[15] for ball lightning in our experiments. □

Received 27 July 1990; accepted 7 January 1991.

1. *Science of Ball Lightning (Fire Ball)* (ed. Ohtsuki, Y. H.) (World Scientific, Singapore, 1989).
2. Barry, J. D. *J. atmos. terr. Phys.* **30,** 313–317 (1968).
3. Ofuruton, H. & Ohtsuki, Y. H. *Nuovo Cim.* **13C,** 761–768 (1990).
4. Kapitza, P. L. *Dokl. Akad. Nauk. SSSR* **101,** 245–248 (1955).
5. Babat, G. I. *J. Inst. electr. Eng.* **94,** Part 3, 27–37 (1947).
6. Hamilton, C. W. *Nature* **188,** 1098–1099 (1960).
7. Richie, D. J. *J. Inst. electr. Eng.* **9,** 202–206 (1963).
8. Silberg, P. A. *J. appl. Phys.* **35,** 2264–2265 (1964).
9. Powel, J. R. & Finkelstein, D. *Am. Sci.* **58,** 262–280 (1970).
10. Brand, W. *Der Kugelblitz* (Henri Grand, Hamburg, 1923).
11. Barry, J. D. *Ball Lightning and Bead Lightning* (Plenum, New York, 1980).
12. Kosarev, E. L. *et al. Soviet Phys. Tech. Phys.* **13,** 1477–1479 (1969).
13. Kosarev, E. L. & Serezhkin, Yu. G. *Soviet Phys. Tech. Phys.* **19,** 229–233 (1974).
14. Zheng, X. H. *Phys. Lett.* **A148,** 463–469 (1990).
15. Handel, P. H. in *Science of Ball Lightning (Fire Ball)* (ed. Ohtsuki, Y. H.) 254–259 (World Scientific, Singapore, 1989).

ACKNOWLEDGEMENTS. The authors thank Prof. K. Kohra and Mr. H. Ara for their help. We also thank Tsukuba Institute of Science and Technology.

The 154-day and related periodicities of solar activity as subharmonics of a fundamental period

T. Bai & P. A. Sturrock

Center for Space Science and Astrophysics, Stanford University,
Stanford, California 94305, USA

IN 1984 a periodicity of 154 days was found in the record of solar flare activity[1] from 1980 to 1983. Since then, the same periodicity has been found in many different measures of solar flare activity during cycle 21 (soft X-ray peak flux[1]; hard X-ray emission[2,3]; Hα flare activity[4]; microwave peak flux[5]; production of interplanetary electrons[6] and protons[7]; 10-cm radio flux[8]) as well as in sunspot area[9,10]. The cause of this 154-day periodicity, which has also been discovered in solar cycles 19 and 20 (refs 7, 11) remains unknown, but a suggestion that it was related to enhanced flare activity in certain longitude bands has been ruled out[3]. Here we show that periodicities of 51, 78, 104 and 129 days, in addition to the 154-day period, can often be detected in flare and sunspot records. These periods are close to integral multiples (by factors of 2, 3, 4, 5 and 6) of 25.8 days, suggesting that they are subharmonics of a fundamental period.

Although there is evidence that the 154-day periodicity was in operation during solar cycle 20, in most cases, for the same kind data, the 154-day power for cycle 20 is weaker than the 154-day power for cycle 21 (refs 4, 5, 8, 11). The strongest evidence that the 154-day periodicity was in operation during cycles 19 and 20 is found in the rate of flares that produce interplanetary energetic protons[7]. When the proton-flare rates of cycles 19 (1955–1965) and 20 (1966–1975) are analysed together, it is found that the 154-day power comes mainly from the 1958–1971 interval. The 154-day peak for this interval is at a 99.8% confidence level. Bai and Cliver[7] showed that the phase of the 154-day periodicity had shifted by about 180° when the periodicity resumed in the proton-flare occurrence rate for the interval from April 1978 to August 1983. (See ref. 12 for a discussion of phase coherence.)

There is evidence from analyses of the daily sunspot number[12] and the daily sunspot area (ref. 13; and M. Carbonell and J. L. Ballester, preprint) that the 154-day periodicity was present before solar cycle 19. In addition, the power spectrum of the equatorial solar diameter measured during the interval 1683–1718 shows 6σ peak at 155 days[14]. Thus it seems that the appearance of this periodicity is not a one-time chance event but represents a fundamental property of the Sun. The values of period found by various authors fall in the range from 150 to 158 days.

We calculated the statistical significance of periodicities as follows. The distribution function of power follows an exponential form at low values of power, and when the power is properly normalized, the probability p of obtaining a peak with a height h at a given frequency by chance is simply $p = \exp(-h)$ (ref. 7 and references therein). The probability of obtaining such a high peak at one or more of N independent frequencies is

$$p = N \exp(-h) \tag{1}$$

Here $N = (f_2 - f_1)T$, where $(f_2 - f_1)$ is the frequency interval of search and T is the time interval for the data run.

To determine the time interval during which the periodicity operates, we use the maximum-likelihood method. The logarithmic likelihood function is defined as

$$m(A, P, \phi; n) = \sum_{i=1}^{n} X_i \ln[1 + A \cos 2\pi(t_i/P + \phi)] \tag{2}$$

where X_i is the time series normalized such that the average is unity, P is period and ϕ is phase. Using the power spectral analysis, we can determine the period and the phase angle, from which in turn we can determine the amplitude A that maximizes the likelihood function. Once we know the values of A, P and ϕ that maximize the likelihood, we can study the logarithmic likelihood as a function of n (time), because it is cumulative. In a cycle of period P for which the signal displays the periodic modulation, the X_is are likely to be greater than 1 when $\ln[1 + A \cos 2\pi(t_i/P + \phi)] > 0$ (or equivalently,

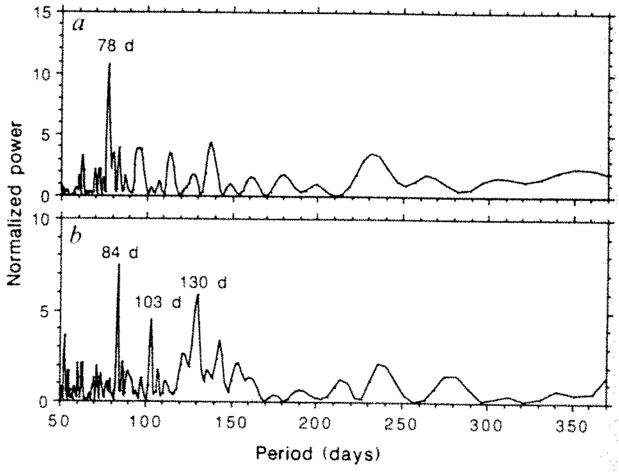


FIG. 1 *a*, Normalized power spectrum of daily sunspot-area measurements from 10 November 1971 to 14 July 1978. *b*, Normalized power spectrum of the rate of flares with CFI > 6 for solar cycle 20.

$[1+A\cos 2\pi(t_i/P\phi)]>1$), and X_i are likely to be smaller than 1 when $\ln[1+A\cos 2\pi(t_i/P+\phi)]<0$. Therefore, when a cycle exhibiting the periodicity is completed, the logarithmic likelihood function increases; otherwise it decreases. We identify an interval of consecutive cycles for which the logarithmic likelihood function increases as an interval for the periodicity. This is an objective way of finding time intervals for which the periodicity is in operation. This method is a generalization of the method given by Bai and Cliver[7].

Bai[15] analysed the rate of flares selected by the comprehensive flare index (CFI) from compilations of Dodson and Hedeman[16,17] for cycle 19, and found that the strongest periodicity was at 50.9 (±0.7) days. (Hereafter the range of period indicates the full width at half maximum.) This periodicity is significant at a 98.5% level[15]. We find that the 51-day periodicity was evident from November 1956 to September 1960. The 51-day periodicity is found to be as strong as the 154-day periodicity in the power spectrum of daily sunspot-area measurements for cycle 21 (ref. 18). Also there is a 51-day peak in the power spectrum of the whole-disk 10-cm radio flux measurement for the interval 1948–1987[9]. Furthermore, in the power spectrum of the γ-ray flare rate for cycle 21 (ref. 6), the 51-day peak is the strongest after the 154-day peak in the period range 23–1,000 days. As 51 days is about one-third of 154 days, a connection between the two periodicities has been proposed[15].

In the power spectrum of the rate of flares selected by microwave peak fluxes for solar cycle 20, the peak at 78 days is the strongest in the 30-500-day interval[5]. During the 1971–1977 interval, the proton flare rate did not exhibit the 154-day periodicity (compare ref. 7). We analysed the power spectrum of the daily sunspot-area measurements and found a strong peak at 77.8 (±1.0) days (Fig. 1*a*), which is half of 154 days. The 78-day peak has a power of 10.7. The probability of obtaining such a peak by chance at one of the 200 frequencies that we studied is $P=200\exp(-10.7)=0.005$. The 78-day periodicity is found to be present from November 1971 to June 1978, roughly the same interval as that during which the proton flare rate did not exhibit the 154-day periodicity. Thus, in late 1971 the 154-day periodicity switched to a periodicity with half the period, and then in 1978 it switched back to the 154-day periodicity. The time interval during which the 154-day periodicity was switched to the 78-day periodicity corresponds to 31 cycles of 78 days. This odd number of cycles may be the reason for the 180° phase shift of the 154-day periodicity of the proton flare rate.

Figure 1*b* shows the normalized power spectrum of the rate of flares with CFI > 6 for cycle 20 (flare compilations of refs 17, 19). The three strongest peaks are at 84, 130 and 103 days, in that order. The power for the 84-day periodicity is found to come mainly from the interval from August 1965 to December 1975. The peak is at 83.8 days. The power for the 130-day periodicity comes mostly from the two intervals November 1967 to January 1971 (9 cycles) and March 1973 to July 1978 (15 cycles), and if we confine our analysis to the interval from November 1967 to July 1978, the peak is at 129.9 (±0.7) days. The power for the 103-day periodicity comes mainly from the interval between November 1969 and February 1976, and if we confine our analysis to this interval, the peak is at 104.4 (±1.6) days. The normalized power of the peak in Fig. 1*b* is 4.55; the probability of obtaining such a peak by chance at a given frequency is $p=\exp(-4.55)=0.01$. The probability of obtaining such a peak anywhere between 30 and 230 nHz (50–370 days) is large, but the probability for finding such a peak at a value near four times 25.8 days (in the range 100–105 days, two-thirds of the range 150 to 158 days) is only 6%. The 130-day peak has a normalized power of 5.91, and the probability of obtaining such a peak near five times 25.8 days (125–132 days) is about 1%.

Figure 2 summarizes the power spectral analysis of CFI flares (1955–1979), SMM flares (flares with peak rates >1,000 counts per s, measured by hard X-ray on the Solar Maximum Mission, 1980–1984), daily sunspot-area measurements (1971–1985) and proton flares (1955–1985). These data sets, which cover all or parts of cycles 19–21, have been analysed in detail recently[3,7,9,16]. Proton flares cover all three cycles, and CFI flares and SMM flares together cover three solar cycles. We cannot help but notice that, except for 84 days, all the periods are equal to integral multiples of 25.8 days within the error bars quoted earlier. Integral multiples of the 25.0–26.3-day interval cover about 5% of the 50–370-day interval. The probability that four out of the five additional periodicities (in addition to the 154-day period) in Fig. 2 fall by chance in these narrow intervals is $p=5\times(0.05)^4=3.1\times10^{-5}$.

A nonlinear dynamical system with a periodic forcing term can show periodic behaviour at subharmonic frequencies (that is, at periods that are integral multiples of the fundamental periods). For instance, one of the best-studied nonlinear systems is that described by Duffing's equation,

$$\frac{d^2x}{dt^2}+k\frac{dx}{dt}+x^3=B\cos\omega t \qquad (3)$$

where k and B are constants. For certain values of k and B, this system can exhibit periodic behaviour at subharmonics, and for other values it can show chaotic behaviour[20,21].

According to this interpretation, solar activity involves a nonlinear response to a solar 'clock' with a period of about 25.8 days. Determining the nature of this clock then becomes the key to understanding the 154-day and related periodicities exhibited by solar activity. Evidence for the 25.8-day clock and its interpretation will be discussed elsewhere[22]. □

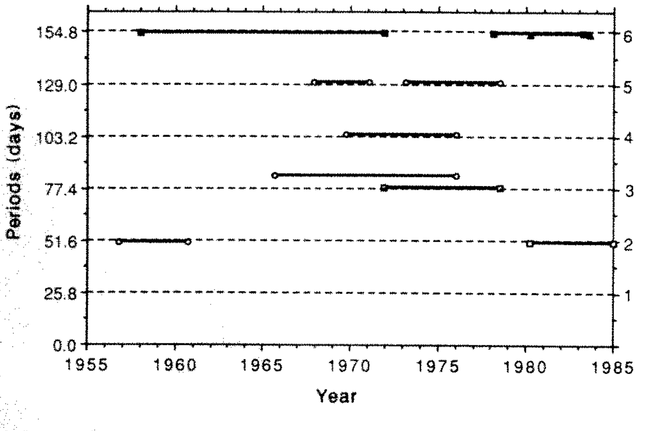


FIG. 2 Various periodicities as integral multiples of 25.8 days. The horizontal bars indicate the time intervals for various periods. ■, Proton flares; ▲, SMM flares; ○, CFI flares; □ sunspot area.

Received 23 October 1990; accepted 17 January 1991.

1. Rieger, E. *et al. Nature* **312,** 623–625 (1984).
2. Dennis, B. R. *Solar Phys.* **100,** 465–490 (1985).
3. Bai, T. & Sturrock, P. A. *Nature* **327,** 601–604 (1987).
4. Ichimoto, K., Kubota, J., Suzuki, M., Tohmura, I. & Kurokawa, H. *Nature* **316,** 422–424 (1985).
5. Bogart, R. S. & Bai, T. *Astrophys. J.* **299,** L51–L55 (1985).
6. Droege, W. Gibbs, K., Grunsfeld, J. M., Meyer, P. & Newport, B. J. *Astrophys. J. Suppl.* **73,** 279–283 (1990).
7. Bai, T. & Cliver, E. W. *Astrophys. J.* **363,** 299–309 (1990).
8. Kile, J. N., Cliver, E. W. & Fougere, P. F. *Astrophys. J.* (in the press).
9. Pap, J., Tobsika, W. K. & Bouwer, S. D. *Solar Phys.* **129,** 165–189 (1990).
10. Lean, J. L. & Brueckner, G. E. *Astrophys. J.* **337,** 568–578 (1990).
11. Ozguc, A. & Atac, T. *Solar Phys.* **123,** 357–365 (1989).
12. Wolff, C. L. *Astrophys. J.* **264,** 667–676 (1983).
13. Lean, J. L. *Astrophys. J.* **363,** 718–727 (1990).
14. Ribes, E., Merlin, Ph., Ribes, J.-C. & Barthalot, R. *Annales Geophys.* **89-04,** 321–329 (1989).
15. Bai, T. *Astrophys. J.* **318,** L85–L91 (1987).
16. Dodson, H. W. & Hedeman, E. R. *World Data Center for Solar-Terrestrial Physics Report* **UAG-14** (1971 NOAA, Boulder).
17. Dodson, H. W. & Hedeman, E. R. *World Data Center for Solar-Terrestrial Physics Report* **UAG-52** (NOAA, Boulder, 1975).
18. Bouwer, S. D., Donnelly, R. F. & Pap, J. in *Proc. XXVIII COSPAR* (Hague, The Netherlands, in the press).
19. Dodson, H. W. & Hedeman, E. R. *World Data Center for Solar-Terrestrial Physics Report* **UAG-80** (NOAA, Boulder, 1981).

20. Thompson, J. M. T. & Stewart, H. B. *Nonlinear Dynamics and Chaos* (Wiley, New York, 1986).
21. Ueda, Y. in *New Approaches to Nonlinear Problems in Dynamics* (ed. Holmes, J. P.) 311–322 (Philadelphia, SIAM, 1981).
22. Sturrock, P. A. & Bai, T. *Bull. Am. astr. Soc.* **22,** 1225 (1990).

ACKNOWLEDGEMENTS. This research was supported by NASA and the Office of Naval Research.

Latex elastomer with a permanently hydrophilic surface

Isao Noda

The Procter & Gamble Company, Miami Valley Laboratories,
PO Box 398707, Cincinnati, Ohio 45239-8707, USA

WATER wettability is important in many applications of polymeric materials, including fabrics, printing and biomedical uses[1,2]. Various surface-modification techniques are available to convert the intrinsically hydrophobic surfaces of plastic to water-wettable ones, by incorporating chemically polar functional groups at the surface[4–7]. Although such chemically induced surface hydrophilicity can be relatively long-lived while the substrates remain rigid, increases in the mobility of surface molecules, for example, due to increasing temperature, can cause rapid loss of hydrophilicity, driven by the tendency of surfaces to minimize their free energy[2–5]. As the polar groups are not often bound to the polymer matrix (usually being instead free surfactant molecules), they may also be flushed from the surface by repeated exposure to water. Here I report the preparation of permanently water-wettable elastomeric films from a latex synthesized by polymerization of monomers in the presence of an amphiphilic block copolymer. By migration of the hydrophilic segments to the surface during film formation, the film is rendered essentially completely wettable by water. Applications may include flexible coatings that can introduce spatially selective wettability to solid surfaces, for example one-side wettable perforated films used for bandages and disposable diapers[8].

Figure 1*a* shows a water droplet placed on a film of synthetic rubber. The water contact angle exceeds 90°, indicating the film surface is hydrophobic. Similar water-repelling behaviour is observed for most natural or synthetic rubbers, unless surfactants are added to the system to lower the surface tension of the water. Such reduction of the liquid surface tension certainly results in an improvement of the apparent wettability of the surface, but surfactant-induced wetting is in many ways qualitatively different from the spreading of water over truly hydrophilic solid surfaces.

In particular, the effect of surfactant treatment is usually temporary. The wettability diminishes once the surface is rinsed with excess water. Migration of surfactants away from the surface to the bulk interior or to other nearby surfaces can also occur. Furthermore, in many applications (such as printing, coating and biomedical applications), it is often desired to make only a part of the available solid surfaces water-wettable while keeping the rest of the system dry. For such selective, spatially resolved wetting, the distinction between facilitating wetting by making the surface intrinsically hydrophilic or by simply reducing the liquid's surface tension becomes very important.

Figure 1*b* shows a water droplet on the permanently hydrophilic surface of a film made from the new latex that I have developed. The latex was prepared by polymerizing a mixture of monomers (40 parts styrene to 60 parts butadiene) dispersed in water. An amphiphilic block copolymer, consisting of a polyethylene oxide segment of relative molecular mass (M_r) 1,365 attached to a short-chain polybutadiene ($M_r = 480$), was used as an emulsifying agent instead of a typical low-molecular-weight surfactant[8]. Each latex particle is believed to be covered by a layer of the block copolymer, and a substantial fraction of the reactive polybutadiene segment is probably grafted to the latex core during the emulsion polymerization. The latex exhibits an exceptionally high colloidal stability. Extensive dialysis of the latex, even for days, diminishes neither the colloidal stability nor the capability of the latex to form a hydrophilic film.

When the latex is dried, the soft particles fuse together to form a continuous rubbery film. During this stage, redistribution of the block copolymer apparently occurs such that there is a substantial surface excess concentration of the ethoxylate groups (this was detected by an infrared technique). There is a corresponding marked increase in the surface hydrophilicity of the resulting rubber film. A water droplet placed on the film readily spreads over the entire surface (Fig. 1*b*). The advancing water contact angle is so small (well below 10°) that a precise measurement is difficult. Practically speaking, the rubber film is completely water-wettable.

Although the driving force for the surface accumulation of the amphilic block copolymer has not yet been identified, it is likely to be of entropic origin associated with the large size of the polymeric amphiphile, which somehow compensates for the energetic penalty of bringing the polar, high-energy component to the surface. Ageing of the surface-hydrophilic rubber film in dry air at room temperature for several months did not alter the water wettability. The surface hydrophilicity is equally stable at elevated temperatures (>60 °C). If the surface hydrophilicity is due to a simple kinetic effect associated with trapping of hydrophilic constituents near the surface during the formation of the film, prolonged exposure to dry air should eventually drive such moieties away from the surface into the bulk phase[2–5]. The surface hydrophilicity of this material, however, seems quite stable.

Several experiments were conducted to show that the observed water wettability indeed arises from the increased surface hydrophilicity of the rubber film and not from the lowering of the surface tension of the water droplet by residual surfactants. Rinsing with excess running water is usually sufficient to remove most of the surfactant from the surface of an ordinary rubber,

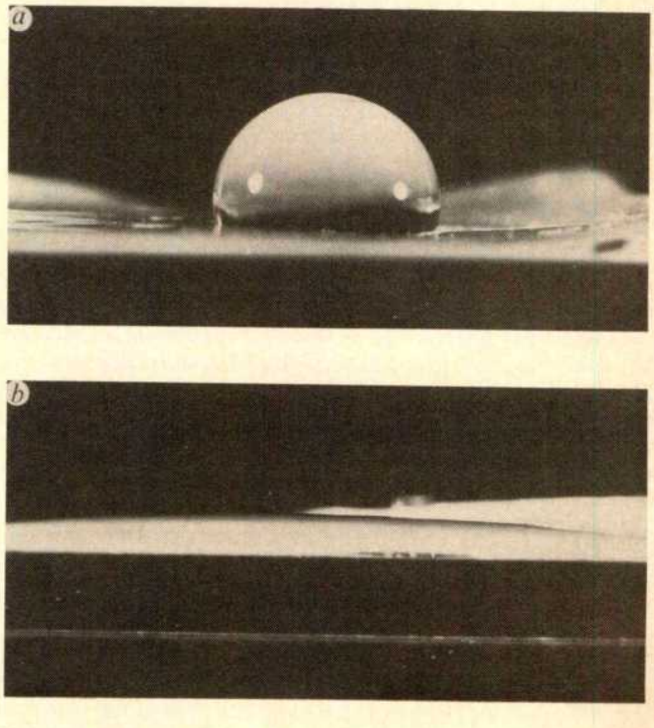


FIG. 1 *a*, A droplet of water placed on a film of ordinary styrene–butadiene rubber. The water beads up, indicating that the surface is hydrophobic. *b*, Water droplet spreading over the hydrophilic surface of the new elastomer film.

making it hydrophobic. This surface-hydrophilic rubber film, however, remained completely water-wettable even after many days of rinsing. The amphiphilic block copolymer responsible for the wettability is attached quite firmly (probably covalently) to the rubber surface. The bulk of the material remained totally hydrophobic: there was no detectable swelling of the film by water.

Further evidence that the amphiphilic block copolymer is attached to the rubber surface and not dissolved in the water is obtained by transferring water that has been spread over the hydrophilic rubber onto an ordinary hydrophobic film. The water beads up in a manner similar to that shown in Fig. 1*a*, indicating that the surface tension of the water has not been substantially reduced. When it is placed back on the hydrophilic rubber, the same water spreads again. This procedure can be repeated many times, and demonstrates that the spreading of water over the rubber film is due to the surface hydrophilicity of the film rather than lowering of the liquid's surface tension. □

Received 3 December 1990; accepted 17 January 1991.

1. Ruckenstein, E. & Gourisankar, S. V. *J. Colloid Interface Sci.* **101,** 436–451 (1984).
2. Ratner, B. D., Weathersby, P. K., Hoffman, A. S., Kelly, M. A. & Scharpen, L. H. *J. appl. Polymer. Sci.* **22,** 643–664 (1978).
3. Sharma, A. K., Millich, F. & Hellmuth, E. W. *J. appl. Polymer. Sci.* **26,** 2205–2210 (1981).
4. Yasuda, H., Sharma, A. K. & Yasuda, T. *J. Polymer Sci. Polymer Phys. Ed.* **19,** 1285–1291 (1981).
5. Briggs, D., Rance, D. G., Kendall, C. R. & Blythe, A. R. *Polymer* **21,** 895–900 (1980).
6. Nuzzo, R. G. & Smolinski, G. *Macromolecules* **17,** 1013–1019 (1984).
7. Rasmussen, J. R., Stedronsky, E. R. & Whitesides, G. M. *J. Am. chem. Soc.* **99,** 4736–4745 (1977).
8. Noda, I. US Patent 4,735,843 (1988).

Ice-core record of oceanic emissions of dimethylsulphide during the last climate cycle

M. Legrand*, C. Feniet-Saigne*, E. S. Saltzman†, C. Germain†, N. I. Barkov‡ & V. N. Petrov‡

* Laboratoire de Glaciologie et Géophysique de l'Environnement, BP 96, 38402 St Martin d'Hères, Cedex, France
† Rosenstiel School of Marine and Atmospheric Science, University of Miami, 4600 Richenbacker Causeway, Miami, Florida 33149, USA
‡ Arctic and Antarctic Research Institute, 38 Beringa Street, Leningrad 199226, USSR

THE Vostok ice core in Antarctica has provided one of the longest climate records, enabling the stable-isotope, major-ion and gas composition of the atmosphere to be reconstructed over many thousands of years. Here we present depth profiles along this core of methanesulphonate and non-seasalt sulphate (produced by the atmospheric oxidation of dimethylsulphide), which provide the first historical record of biogenic sulphur emissions from the Southern Hemisphere oceans over a complete glacial–interglacial cycle (160 kyr). Those measurements confirm and extend some previous observations made on a very limited data set from the Dome C ice core in Antarctica, which indicated increased oceanic emissions of dimethylsulphide during the later stages of the glacial period, compared with the present day[1]. The observed glacial–interglacial variations in methanesulphonate and non-seasalt sulphate confirm that the ocean–atmosphere sulphur cycle is extremely sensitive to climate change.

During initial attempts to remove surface contamination from the outer part of the ice-core sections, we washed the core with ultrapure water at a temperature of ~20 °C. This method has been used successfully for the analysis of mineral species[2], and to study methanesulphonate (MSA) content over the past 30 kyr in the Dome C ice core[1]. Vostok data obtained using this procedure are compared with Dome C data for the same climate periods (Table 1). Although the non-seasalt (n.s.s.) sulphate increases from stage A to stage B by a factor of two in both cases, the MSA content at Vostok remains fairly constant during the climate change whereas the Dome C core exhibits an increase by a factor of five from the warm to the cold stage. Such a difference is difficult to understand because Vostok (located 600 km from Dome C on the high Antartic plateau) has similar geographical and meteorological characteristics (elevation, mean annual temperature). Laboratory experiments indicate that extensive rinsing of Vostok ice leads to mobility of MSA, which does not occur with other ions. This effect may be related to the fact that the Vostok core was drilled with kerosene drilling fluid, whereas the Dome C core was drilled dry. In addition, the Vostok core sections were stored for 24 hours at −3 °C before washing, to prevent breakage of the core.

TABLE 1 Non-seasalt sulphate and MSA in the Vostok and Dome C ice cores

	Dome C		Vostok	
	MSA ($ng\ g^{-1}$)	n.s.s. SO_4^{2-} ($ng\ g^{-1}$)	MSA ($ng\ g^{-1}$)	n.s.s. SO_4^{2-} ($ng\ g^{-1}$)
Holocene	2.5 (W)	70 (W)	4 (W)	108 (W)
(Stage A, 0–10 kyr BP)			5 (L)	102 (L)
Glacial age	13 (W)	136 (W)	5 (W)	230 (W)
(Stage B, 18–30 kyr BP)			31 (L)	222 (L)

W, data obtained by washing the ice-core sections. L, data obtained by subcoring with a lathe (see text). Vostok data, this work; Dome C data, ref. 2.

Subsequently, we designed an ice-core lathe equipped with a stainless-steel knife to mechanically remove the outer part of the core (until 95% of the volume was removed). The MSA content of successive fractions from the outer to the inner part of the core (not reported here) is constant, indicating the reliability of the procedure. Using this method, the increase in MSA during the glacial age in the Vostok core (Table 1) is similar in magnitude to that at Dome C. We prepared 47 ice sections in this manner, each representing approximately six years of accumulation. These sections were cut from the core at roughly 40-m intervals over the length of the 2,080-m core[3].

For each sample, the concentrations of Na^+, Ca^{2+}, SO_4^{2-} and $CH_3SO_3^-$ were determined by ion chromatography. MSA and sulphate were determined using an AS5 separator column, a gradient pump system (Dionex 4000i) and an integrator with an accuracy of 5% (at the 95% confidence level). Cations were measured using working conditions reported elsewhere[4]. In a previous Vostok ice-core study[5], it was established that the calcium content mainly reflects the terrestrial input to the Vostok ice and that it is feasible to estimate the marine component of Na^+ (denoted Na_m^+) from determinations of Ca^{2+} and Na^+. It is then possible to calculate the content of non-seasalt sulphate by subtracting seasalt sulphate from the total sulphate.

The profile of n.s.s. sulphate (Fig. 1*b*) shows moderate glacial-interglacial changes from 98–102 $ng\ g^{-1}$ during interglacial stages (G and A, respectively) to 208–240 $ng\ g^{-1}$ during cold stages (B, D and H) (see Table 2). This picture is very similar to that from previously published data[5,6]. Between 1,200 and 1,300 m three samples exhibited unusually high levels of n.s.s. sulphate (>400 $ng\ ml^{-1}$). By comparison with data previously obtained at similar depth[5], it seems that these high concentrations are not related to the long-term pattern of sulphate deposition. Instead, they reflect short-term changes associated with volcanic eruptions which sporadically perturb the background level of sulphuric acid in the Vostok precipitation[7]. At these three levels no significant changes in the MSA content is observed. We therefore neglect these values here. The variations in the MSA profile, like those of n.s.s. sulphate, strongly correlate with climate conditions, changing from 5 $ng\ g^{-1}$ during inter-

TABLE 2 Mean concentrations of MSA and n.s.s. SO_4^{2-} in the Vostok core

Stage	Depth (m)	MSA ($ng\ g^{-1}$)	n.s.s. SO_4^{2-} ($ng\ g^{-1}$)	R (%)	MSA′ ($ng\ g^{-1}$)	n.s.s. $SO_4^{2-\prime}$ ($ng\ g^{-1}$)	S'	$Q_{s'}$
A	0–275	5±2	102±11	5±1.5	5±2	102±11	107±13	1
B	325–550	31±3	222±7	14±1.5	20±2	146±5	166±5	1.55
C	575–850	22±6	168±14	13±4	16±4	120±9	136±10	1.27
D	875–975	32±2	242±17	13±1	21±1	162±9	183±9	1.70
E	1,000–1,475	13.0±4	139±20	10±4	11±3	109±18	120±17	1.1
F	1,500–1,600	19±3	148±7	13±2	13±2	105±5	118±7	1.1
G	1,625–1,875	5.5±3	98±17	5.4±2	5.5±3	100±11	106±13	1.0
H	1,925–2,080	25±1	208±50	12.5±3	16±1	136±31	152±30	1.4

R is the weight ratio of MSA to n.s.s. SO_4^{2-}. Primed values MSA′ and n.s.s. $SO_4^{2-\prime}$ are concentrations corrected for variations in accumulation rate. S' is the sum of MSA′ and n.s.s. $SO_4^{2-\prime}$ and Q' is the ratio of S' in each stage to S' in stage A.

glacial stages (A and G) to 25–31 ng g^{-1} during very cold stages (B, D and H).

One of the notable features of the record is the similarity of the general trends in MSA and n.s.s. sulphate. Because oceanic sulphur is an important source of MSA, this covariance strongly suggests that n.s.s. sulphate in the precipitation over the central Antarctic is primarily derived from oceanic emissions. It is also possible that sources of non-marine sulphate covary with the marine component. Given recent evidence regarding productivity limitation by aerosol-transported nutrients such as iron[8,9], it is perhaps possible that the marine emissions of dimethylsulphide (DMS) from the southern ocean and the long-range transport of continental-derived sulphate might correlate over long timescales and large variations in climate.

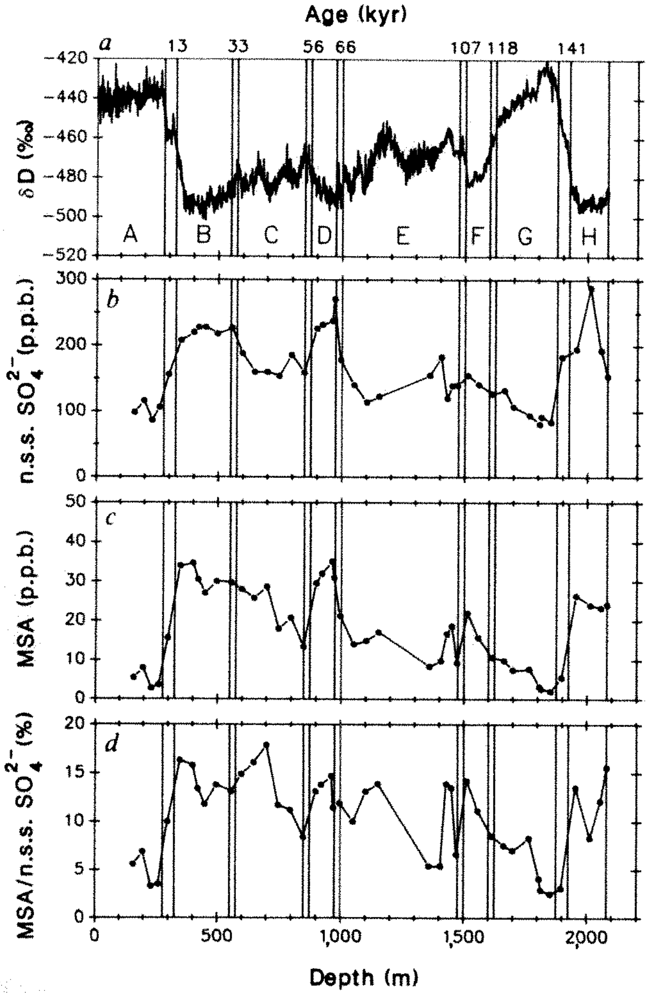


FIG. 1 Depth profiles of *a*, δD, *b*, non-seasalt sulphate, *c*, MSA and *d*, the ratio of MSA to non-seasalt sulphate in the Vostok ice core. Upper and lower stage boundaries are marked by vertical lines. (δD is deuterium isotope profile from ref. 3.)

The relative magnitude of the glacial–interglacial changes in MSA are greater than those of n.s.s. sulphate, resulting in a decrease in the ratio R of MSA to n.s.s. sulphate during warming. During cold stages R is high, with values reaching 14%, and during warm stages R reaches a minimum of 3%. In the present atmosphere, there is a systematic trend of R in marine aerosols, from low ratios in the low and mid-latitudes (2–10%, ref. 10) towards higher ratios at higher latitudes (15–100%; ref. 11). This effect is presumably atmospheric, related to the different temperature-dependence of OH addition and H-atom abstraction in the oxidation of DMS by OH (ref. 12). It is presumed that these different pathways give rise to the two principal products of DMS oxidation, MSA and SO_2 (which is subsequently converted to sulphate). The decrease in R during warming might therefore reflect either increasing temperature in the DMS source region, or a change in the primary DMS source region, with increasing input from long-distance transport from the lower latitudes. Here, we make the implicit assumption that the basic features of the aerosol size distributions of MSA and n.s.s. sulphate (and hence their transport and depositional characteristics) are relatively constant for different climates. This point may bear further investigation in view of recent data indicating that the size distribution of MSA may be dependent on that of the preexisting aerosols[13].

Other possible explanations for the generally low ratios observed in the high Antarctic plateau (for both glacial and interglacial conditions) require the input of a significant non-marine sulphate component. Stratospheric input has historically been considered a possible contribution to the sulphate budget of central Antarctica, but there is now general agreement that this contribution is weak except after sporadic major volcanic eruptions[14–16]. Input of continental-derived sulphate is another possibility, as mentioned above. A significant component of the sulphate present during glacial times, at least, is ionically balanced by calcium and the possibility exists that this sulphate is terrestrially derived. During glacial stages (B, C and D) calcium concentrations in our samples vary widely (more than a factor of three, from 13 to 52 ng g^{-1}), but the ratio R remains essentially constant. This implies that this sulphate was marine in origin and was neutralized in the atmosphere by reaction with calcium carbonate of terrestrial origin.

Because the partitioning between MSA and n.s.s. sulphate seems to be modulated by climate, it is useful to examine the total oxidized sulphur, which we presume to be the sum of MSA and n.s.s. sulphate (Table 2) instead of n.s.s. sulphate alone as in previous studies[5,6]. The higher concentrations of n.s.s. sulphate and MSA found in cold stages are partly due to the reduced accumulation rates during this time. To obtain a more representative picture of the actual atmospheric concentrations in the past, we have corrected concentrations in ice using a method presented elsewhere[6]. We have also assumed that MSA and n.s.s. sulphate have similar deposition processes because both MSA and n.s.s. sulphate are present in the atmosphere as fine particles[10]. The corrected data, shown in Table 2, suggest that the oxidation products of DMS were constant during interglacial stages (stage A and G), but were enhanced significantly during the second part of the glacial age (stage B, C and D). Assuming that inputs of MSA and n.s.s. sulphate over the high Antarctic plateau are related largely to marine DMS emissions at mid-latitudes, our data suggest enhanced productivity from the DMS-producing portion of the oceanic biota between 18 and 70 kyr BP (before present). Whether this increase represents a real increase in oceanic productivity or simply an ecological shift favouring DMS producers is not known. Some workers have suggested that marine sediment cores show evidence for increased productivity between 15 and 70 kyr BP in upwelling areas[17], but this finding remains controversial.

Clearly, the ocean-atmosphere sulphur cycle exhibits variability that is intimately related to global climate change. It has been suggested that the biogenic aerosols themselves play an important part in forcing climate change either through direct radiative effects or through changes in cloud albedo and distribution[6,18,19]. It may be possible to detect the effects of such climate forcing using high-resolution ice-core records which document the timing of changes in biogenic sulphur levels relative to those in stable isotopes, CO_2 and other chemical tracers during periods of rapid climate change. These studies are currently in progress. □

Received 19 September 1990; accepted 8 January 1991.

1. Saigne, C. & Legrand, M. *Nature* **330,** 240–242 (1987).
2. Legrand, M., De Angelis, M. & Delmas, R. *Analytica chim. Acta* **156,** 181–192 (1984).
3. Jouzel, J. *et al. Nature* **329,** 403–408 (1987).
4. Saigne, C., Kirchner, S. & Legrand, M. *Analytica chim. Acta* **203,** 11–21 (1987).
5. Legrand, M., Lorius, C., Barkov, N. I. & Petrov, V. N. *Atmos. Envir.* **18,** 1867–1874 (1987).
6. Legrand, M., Delmas, R. J. & Charlson, R. J. *Nature* **334,** 418–419 (1988).
7. Legrand, M. & Delmas, R. J. *Nature* **327,** 671–676 (1987).
8. Martin, J. H. & Fitzwater, S. E. *Nature* **331,** 341–343 (1988).
9. Duce, R. A. *The Role of Air-Sea Exchange in Geochemical Cycling* (ed. Buat-Menard, P.) 497–529 (Reidel, Dordrecht, 1986).
10. Saltzman, E. S., Savoie, D. L., Zika, R. G. & Prospero, J. M. *J. geophys. Res.* **88,** 10897–10902 (1983).
11. Berresheim, H. *J. geophys. Res.* **92,** 13245–13262 (1987).
12. Hynes, A. J., Wine, P. H. & Semmes, D. H. *J. phys. Chem.* **90,** 4149–4156 (1986).
13. Pszenny, A. A. *J. atmos. Chem.* (submitted).
14. Delmas, R. J. *Nature* **299,** 677–678 (1982).
15. Cunningham, W. C. & Zoller, W. H. *J. Aerosol Sci.* **12,** 367–384 (1981).
16. Legrand, M. & Delmas, R. J. *Atmos. Envir.* **18,** 1867–1874 (1984).
17. Sarnthein, M., Winn, K. & Zahn, R. *Abrupt Climatic Change* (eds Berger, W. H. & Labeyrie, L. D.) 311–334 (Reidel, Dordrecht, 1987).
18. Shaw, G. E. *Clim. Change* **5,** 297–303 (1983).
19. Charlson, R. J., Lovelock, J. E., Andreae, M. O. & Warren, S. G. *Nature* **326,** 655–661 (1987).

ACKNOWLEDGEMENTS. Financial support was provided by the Centre National de la Recherche Scientifique and by the NSF. The Vostok ice core was analysed in the framework of a US–France–USSR cooperative agreement.

Amelioration of subsoil acidity by application of a coal-derived calcium fulvate to the soil surface

H. v. H. van der Watt*, R. O. Barnard*, I. J. Cronje†, J. Dekker†, G. J. B. Croft* & Maria M. van der Walt*

* Department of Soil Science and Plant Nutrition, University of Pretoria, 0002 Pretoria, South Africa
† Division of Energy Technology, CSIR, PO Box 375, 0001 Pretoria, South Africa

SUBSOIL acidity is a serious problem in many tropical and subtropical soils[1–3]. The high acidity, low calcium contents and often toxic levels of soluble and/or exchangeable aluminium severely impair plant-root development in these soils[1–3]. The relative immobility of surface-applied liming materials limits their ability to reduce subsoil acidity. Recently, the use of gypsum or phosphogypsum has been advocated as an alternative to lime[3–7]. On the other hand, it is well known that humic substances can mobilize and form complexes with metals in soils[8–11]. Here we report that a newly available, coal-derived calcium-fulvate is highly efficient as a carrier of calcium in the soil profile. Moreover, subsoil pH was considerably higher when calcium-fulvate was applied to the soil surface, than when gypsum, calcium-EDTA, $Ca(OH)_2$ or $CaCO_3$ were applied.

Fulvic acid was obtained from the wet oxidation of coal at elevated pressure and temperature, using a recently patented process[12]. This process leads to the formation of a water-insoluble product (oxicoal, containing ~80% base-soluble 'humic' acids) and water-soluble 'fulvic' acids (10–15% yield). The fulvic acids, separated by filtration from the oxicoal, were neutralized to pH 7.2 with $Ca(OH)_2$.

We called the resulting 'solution' (actually a stable suspension because colloidal particles are usually still present) Ca-fulvate. The suspension could be dried to a fine, dark powder but, in our experiments, it was used without further drying. It contained 4.18% total solids and 11.26 g Ca l^{-1}.

TABLE 1 Relevant properties of experimental soils

Soil	Clay (<2 μm) (%)	pH (H_2O)	pH (1 mol l^{-1} KCl)	CEC* (soil) (cmol$_c$ kg^{-1})	Exch.† bases (% of CEC)	Exch.† Ca (% of CEC)
Avalon	6.0	4.68	3.74	1.7	29.0	11.5
Hutton UP	20.2	5.02	4.08	3.2	39.7	18.8
Hutton PR	35.6	4.70	3.97	4.3	28.3	18.0

* Cation exchange capacity (cmol of charge per kg soil).
† Exchangeable.

The downward movement of Ca in soil was studied using cylinders 1 m long × 68 mm inside diameter. Plastic sheeting was used as lining to allow the easy removal of the entire soil column for segmentation and analysis. Care was taken to eliminate micro-channels which may result in preferential flow paths at the soil-liner boundary. Three soil types were used in our experiments (Table 1). The columns were carefully packed with dry soil and leached with two pore volumes of deionized water as a pretreatment to remove free salts. The following substances were then added in 150-cm^3 suspensions to the surface of the water-saturated soil column: H_2O (control), $CaCO_3$, $Ca(OH)_2$ (freshly prepared), $CaSO_4{\cdot}2H_2O$, Ca-EDTA and Ca-fulvate. In the Ca treatments 1.689 g Ca was added in each case; this is equivalent to 20 t ha^{-1} gypsum or 11.6 t ha^{-1} $CaCO_3$. The columns were leached with deionized water at a flow rate of 55 mm h^{-1} until two pore volumes of leachate had been collected in measured fractions. This flow rate is fairly high, but of course lower than the hydraulic conductivity of the columns. The effect of flow rate on Ca distribution may be important, but was not studied. After cessation of water inflow, the columns were allowed to drain. The drainage water was collected as a final leachate fraction. The soil column encased within the lining could easily be removed without disturbance, and was cut into 100-mm-long segments. The segments were dried at 353 K and weighed. The leachate fractions were analysed for Ca, Mg, Na, K, Fe and Al and the pH determined. The soil segments were analysed for the following: total soluble and exchangeable Ca, Mg, Na and K extractable with 1 mol^{-1} l^{-1} ammonium acetate (pH 7) using a 1 : 10 soil/solution ratio; soluble cations in a 1 : 1

TABLE 2 Calcium derived from surface-applied suspensions and leached to below 150 mm in soil columns

	Ca in excess of control found in:			Percentage of applied Ca leached to:	
Soil and treatment	soil 150–430 mm (mg)	soil 430–920 mm (mg)	leachate (mg)	>150 mm (%)	>430 mm (%)
Avalon					
$CaSO_4$	112	147	205	27.5	20.8
Ca-EDTA	183	190	5	22.4	11.5
Ca-fulvate	339	384	375	65.0	44.9
Hutton UP					
$CaSO_4$	147	0	23	10.1	1.4
Ca-EDTA	321	234	20	34.0	15.0
Ca-fulvate	352	368	268	58.5	37.7
Hutton PR					
$CaSO_4$	183	62	0	14.5	3.7
Ca-EDTA	365	120	0	28.7	7.1
Ca-fulvate	293	267	189	44.3	27.0

TABLE 3 Total cations leached from 1-m long soil columns surface-treated with various Ca compounds for the Hutton soil

	Total cations leached					
Compound added	Ca	Mg	Na (mg)	K	Fe	Al
0	4	2	0	4	0	6
$CaCO_3$	2	1	0	3	0	2
$Ca(OH)_2$	7	2	0	8	0	2
$CaSO_4$	3	2	1	4	1	2
Ca-EDTA	3	10	9	9	27	340
Ca-fulvate	193	78	8	15	1	37

soil/water extract; and Fe and Al extractable with 1 mol l^{-1} KCl using a 1:10 soil/solution ratio. All cations were determined using standardized atomic-absorption spectrometry procedures.

Some of our results are shown in Figs 1 and 2. We consistently found that soil pH increased with depth where Ca-fulvate was applied, but when gypsum was applied, we always found that soil pH became lower in contrast to findings reported elsewhere[3]. The exchangeable-plus-soluble soil Ca at depths below 150 mm and 430 mm in the soil increased dramatically where Ca-fulvate was applied. This effect was much less marked for applications of Ca-EDTA and gypsum, whereas $CaCO_3$ and $Ca(OH)_2$ did not provide Ca to the deeper soil zones, as would be expected considering their low solubilities (the data for $Ca(OH_2)$ and $CaCO_3$ are not shown for the sake of brevity). In Table 2 the percentage of Ca derived from the surface-applied substances and leached beyond the above depths in the soil columns is shown for three of the Ca compounds. Where Ca-fulvate was used, substantial quantities of Ca (11–22% of the Ca applied) were found in the leachate. Because the term fulvic acid refers to a group of substances[10] with molecular weights in the range 500–2,000, this result indicates that at least some fraction of the Ca-fulvate complex is very stable and releases its Ca very slowly or not at all while moving down the soil column. This was confirmed by the pattern of the breakthrough curves, which show a sharp peak after about one pore volume of leachate has been collected following surface application of the Ca-fulvate suspension. Figure 2 shows clearly that Ca-fulvate is effective in supplying Ca to the subsoil.

The higher soil pH which results from the application of Ca-fulvate may be due to one or more of the following reactions: the release of some Ca to the soil solution through the hydrolysis of Ca-fulvate, which occurs continuously as the soluble Ca-fulvate is leached downward; the replacement of exchangeable Al and H by Ca, followed by the removal of Al and H, complexed with the fulvic acid, either by leaching to lower soil horizons or carried away in the leachate. The latter mechanism seems to apply particularly to EDTA, as considerable amounts of H, Al and Fe were found in the leachates from the EDTA treatments (Table 3). Leaching is an essential part of the process for any surface-applied amendment. Furthermore, reactive hydroxyls may also be carried by the fulvate (fulvic acid neutralized to pH 7.2), or as the ion pair $Ca(OH)^+$, which would result in the direct neutralization of soil acidity. The differences in the results for Ca-EDTA and Ca-fulvate may in part be explained by the observation that, in the case of Ca-EDTA, much of the Ca is retained within the top 5 cm of soil (data not shown). The exchangeable acidity replaced by Ca in this way would explain the lowering of the pH deeper in the soil, whereas the more

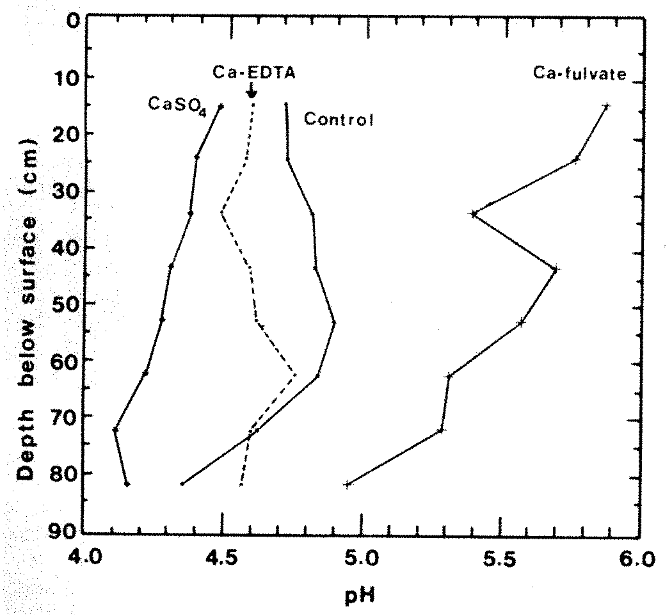


FIG. 1 Soil pH/depth relationships for the Avalon soil following surface applications of $CaSO_4$, Ca-EDTA and Ca-fulvate and leaching.

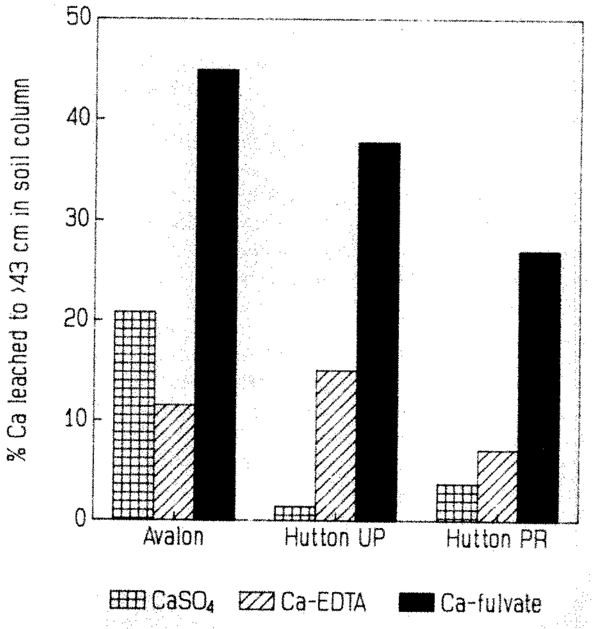


FIG. 2 Diagram showing the percentage of applied Ca from three surface-applied substances leached to a soil depth >43 cm, for three soils.

stable Al-EDTA appears in the leachate (Table 3). In contrast, owing possibly to differences in stability constants and/or reaction rates, the Ca from the Ca-fulvate is released more gradually, or is leached to below the 1 m depth (Table 3).

We conclude that the use of coal-derived Ca-fulvate as an ameliorant for acid and Ca-deficient subsoils is very promising, particularly as only moderate leaching was used in our experiments. But considerable further research is required on aspects such as product optimization and economics, effects on plants and possible groundwater contamination with metal-fulvate complexes. At present we estimate that the product will cost less than 300 dollars per tonne. In practice, use thereof will be determined by crop response and the relevant studies are under way. ☐

Received 11 June; accepted 31 December 1990.

1. Bouldin, D. R. *Cornell Int. Agric. Bull.* No. 34 (1979).
2. Haynes, R. J. *Adv. Agron.* **37,** 249–315 (1984).
3. Shainberg, I. *et al. Adv. Soil Sci.* **9,** 1–111 (1989).
4. Reeve, N. G. & Sumner, M. E. *Agrochemophysica* **4,** 1–6 (1972).
5. Farina, M. P. W. & Channon, P. *Soil Sci. Soc. Am. J.* **52,** 175–180 (1988).
6. Pavan, M. A., Bingham, F. T. & Pratt, P. F. *Soil Sci. Soc. Am. J.* **46,** 1201–1207 (1982).
7. Oates, K. M. & Caldwell, A. G. *Soil Sci. Soc. Am. J.* **49,** 915–918 (1985).
8. Gregor, J. E., Powell, H. K. T. & Town, R. M. *J. Soil Sci.* **40,** 661–673 (1989).
9. Saar, R. A. & Weber, J. H. *Environ. Sci. Tech.* **16,** 510A–517A (1982).
10. Stevenson, F. J. *Humus Chemistry: Genesis, Composition Reactions* (Wiley, New York, 1982).
11. Aiken, R. A., McKnight, D. M., Wershae, R. L. & MacCarthy, P. (eds) *Humic Substances in Soil, Sediment, and Water* (Wiley, New York, 1985).
12. Cronje, I. J. *Process for the Oxidation of Coal.* S. Afr. Patent 88/4770 (1988).
13. Lindsay, W. L. *Chemical Equilibria in Soils* (Wiley, New York, 1979).

Cryptomonad algae are evolutionary chimaeras of two phylogenetically distinct unicellular eukaryotes

Susan E. Douglas*, Colleen A. Murphy*, David F. Spencer† & Michael W. Gray†

* Institute of Marine Biosciences, National Research Council of Canada, 1411 Oxford Street, Halifax, Nova Scotia B3H 3Z1, Canada
† Department of Biochemistry, Dalhousie University, Halifax, Nova Scotia B3H 4H7, Canada

ALTHOUGH it is widely accepted that the plastids of plants and algae originated as endosymbionts[1], the details of this evolutionary process are unclear[2,3]. It has been proposed that in organisms whose plastids are surrounded by more than two membranes, the endosymbiont was a eukaryotic alga rather than a photosynthetic prokaryote[4]. The DNA-containing[5] nucleomorph[6] of cryptomonad algae appears to be the vestigial nucleus of such an algal endosymbiont[7]. Eukaryotic-type ribosomal RNA sequences have been localized to a nucleolus-like structure in the nucleomorph[8]. In support of the hypothesis that cryptomonads are evolutionary chimaeras of two distinct eukaryotic cells, we show here that *Cryptomonas* Φ contains two phylogenetically separate, nuclear-type small-subunit rRNA genes, both of which are transcriptionally active. We incorporate our rRNA sequence data into phylogenetic trees, from which we infer the evolutionary ancestry of the host and symbiont components of *Cryptomonas* Φ. Such trees do not support the thesis[3] that chromophyte algae evolved directly from a cryptomonad-like ancestor.

To identify nuclear-type small-subunit (18S) rRNA genes in a cryptomonad alga, we amplified total *Cryptomonas* Φ DNA by polymerase chain reaction (PCR) using primers specific for nuclear-encoded 18S rRNA sequences[9]. This gave two products, one (Nu) of 1.8 kilobase pairs (kb) and another (Nm) ~200 base pairs (bp) longer. The sequence of the Nu amplification product corresponds to that of the 18S rRNA gene encoded by a 9.5-kb nuclear rDNA repeat, independently cloned from the main DNA band of a Hoechst dye-CsCl density gradient and completely sequenced (D.F.S., unpublished results). The Nm product represents a distinct, novel 18S rRNA gene, which we tentatively assign to the nucleomorph. When applied to total DNA from non-cryptomonad algae, PCR amplification yields only a single product, as expected.

The Nu and Nm sequences (1,773 and 2,039 nucleotides, respectively) are aligned in Fig. 1. Although the primary sequences are very similar (85.2%, excluding variable regions[10] V2 and V4), it is evident that the Nm gene is not simply a variant of the Nu gene, but is structurally distinct. Secondary-structure models (data not shown) demonstrate that both genes encode potentially functional 18S rRNAs, with variable regions confined to portions of the secondary structure previously recognized as divergent among small-subunit rRNAs[11]. There are no obvious structural aberrations to suggest that the Nm gene is an inactive pseudogene; on the contrary, the Nm and Nu secondary structures both contain the complete conserved core[11] that is critical for small-subunit rRNA function[12].

Using the Nm clone to probe Southern blots of *Cryptomonas* total cellular DNA, we found that the Nm and Nu rRNA genes originate from DNA components of widely different base composition (Fig. 2). The Nm probe hybridized to a 7.5-kb *Pst*I restriction fragment that is enriched in a high-(A+T) fraction (fraction 2, Fig. 2) of *Cryptomonas* DNA; this fragment is distinct from the main *Pst*I fragment (>45 kb), tentatively assigned to mitochondrial DNA[13], also found in this region of a Hoechst dye-CsCl density gradient. Cross-hybridization of the Nm probe to the nuclear rDNA repeat is evident in fractions 7–9. The Nm probe did not hybridize to plastid rDNA-containing *Pst*I fragments of 8.6 and 10.0 kb[13] in fractions 4 and 5.

To determine whether the Nm and Nu genes are both expressed, complementary oligodeoxyribonucleotide probes specific for each sequence (A, B and C; Fig. 1) were hybridized to duplicate northern blots. Probe A (Nu-specific) hybridized to a prominent RNA band of ~1.8 kb, in agreement with the size predicted from the DNA sequence (lane 2, Fig. 3). Probe B (Nm-specific) hybridized to an RNA species of 1.45 kb, which is considerably smaller than expected from the gene sequence (lane 3, Fig. 3). A third probe (C, specific for the Nm 5′-terminal region) detected an additional small RNA of ~250 nucleotides (lane 4, Fig. 3). These results not only verify that the Nm gene is transcribed, but also indicate that the mature Nm small-subunit rRNA is discontinuous. Interestingly, the break coincides with an apparent 166-nucleotide insertion in helix E10-11 (ref. 10) contained within variable region V2; this insert must represent a site of post-transcriptional spacer excision. A discontinuity in the V2 region has been described in the case of the mitochondrial small-subunit rRNA of *Tetrahymena pyriformis*[14], but the split Nm rRNA reported here is the first example of a discontinuous nucleocytoplasmic 18S rRNA.

Although proof that the Nm rRNA gene is encoded by nucleomorph DNA must await isolation of a nucleomorph-enriched subcellular fraction and/or *in situ* hybridization experiments using Nm-specific probes, our data strongly support the idea that the cryptomonad nucleomorph contains active eukaryotic-type rRNA genes. Phylogenetic analysis by construction of rRNA trees using both maximum parsimony[15,16] and neighbour-joining[17] methods, shows that the *Cryptomonas* Nm and Nu sequences are evolutionarily distant from one another (Fig. 4). Regardless of the particular tree construction method used, three features of these nuclear small-subunit rRNA trees are consistently observed. (1) The Nm sequence branches as a specific relative of two rhodophyte nuclear 18S rRNA sequences, supporting the hypothesis that the cryptomonad endosymbiont was a primitive red alga[4]; (2) the Nu sequence branches separately from the Nm sequence and in the same assemblage as the amoeboid protozoan *Acanthamoeba castellanii* (in the tree

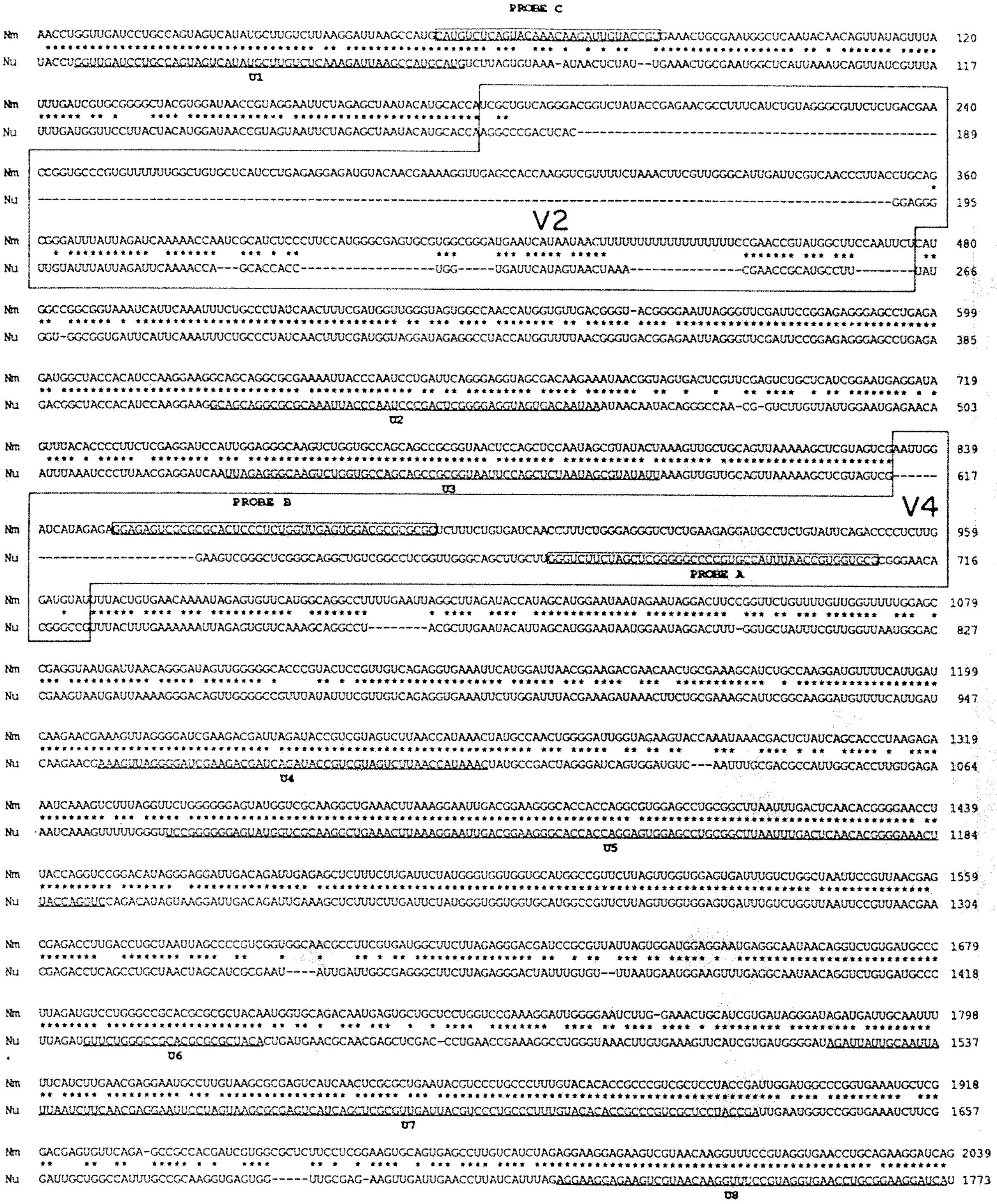


FIG. 1 Alignment of the nuclear (Nu) and putative nucleomorph (Nm) small-subunit rRNA sequences of *Cryptomonas* Φ. The alignment is based on secondary-structure models constructed for the two (data not shown). Dashes indicate apparent deletions and asterisks denote nucleotide identities shared by the two sequences. Principal variable regions (V2, V4)[10] are enclosed by solid lines. Sequences corresponding to the universally conserved regions (U1–U8)[11] that define the functional small-subunit rRNA core are underlined. Positions of complementary oligodeoxynucleotide probes (A, B, C) used in northern hybridizations (see Fig. 3) are indicated by overlining. METHODS. Total cellular DNA was prepared from *Cryptomonas* Φ (ref. 13) and used as a substrate for PCR. Ribosomal RNA genes were amplified[9] using primers containing sequences complementary to the 5′- and 3′-terminal regions of eukaryotic cytoplasmic (18S) small-subunit rRNA (5′-CAACCTGGTTGATCCTGCCAGT-3′ and 5′-CTGATCCTTCTGCAGGTTCACCTAC-3′, respectively) and cloned into pUC18. Both strands were sequenced by the dideoxynucleotide method[22] with synthetic oligonucleotide primers complementary to conserved regions of the small-subunit rRNA molecule. The DNA sequence for the Nu gene was independently generated during analysis of the nuclear rDNA repeat unit (D.F.S., unpublished). A 9.5-kb *Bam*HI restriction fragment corresponding to this repeat was cloned in pT7T3 19U (Pharmacia) and completely sequenced by the chemical degradation method[23].

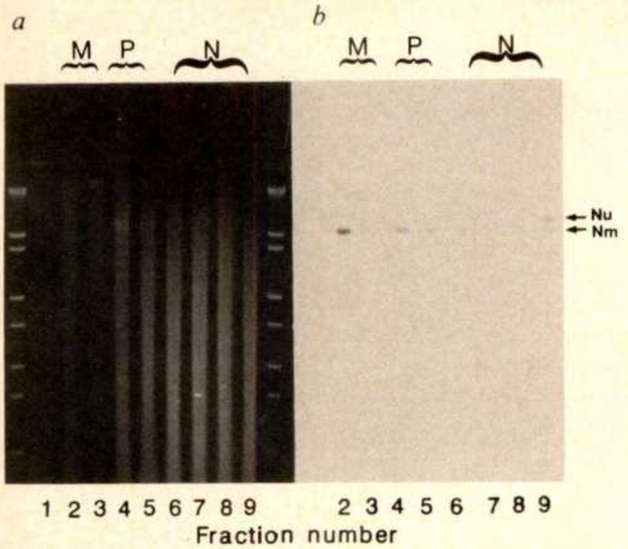


FIG. 2 Detection of rRNA coding sequences in the total DNA from *Cryptomonas* Φ. *a*, Agarose-gel electrophoresis of total DNA fractionated in a Hoechst dye–CsCl density gradient and digested with *Pst*I. Fractions containing mitochondrial (M), plastid (P) and nuclear (N) DNA are indicated. Markers are *Sty*I restriction fragments of bacteriophage λ DNA (24.5, 7.7, 6.2, 3.4, 2.7, 1.89, 1.45 kb). *b*, Autoradiogram of Southern blot hybridized with the Nm clone. The bands corresponding to the nuclear (Nu) and nucleomorph (Nm) rDNA-containing fragments are indicated by arrows. Restriction endonuclease analysis of aliquots from Hoechst 33258 dye–CsCl density gradients, transfer to Zeta Probe membranes (BioRad) and Southern hybridization analysis were as described previously[13].

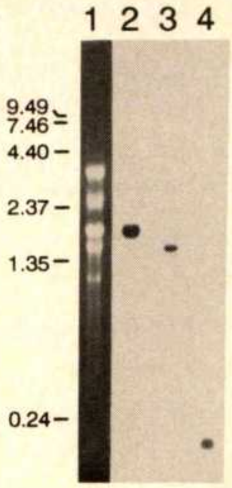
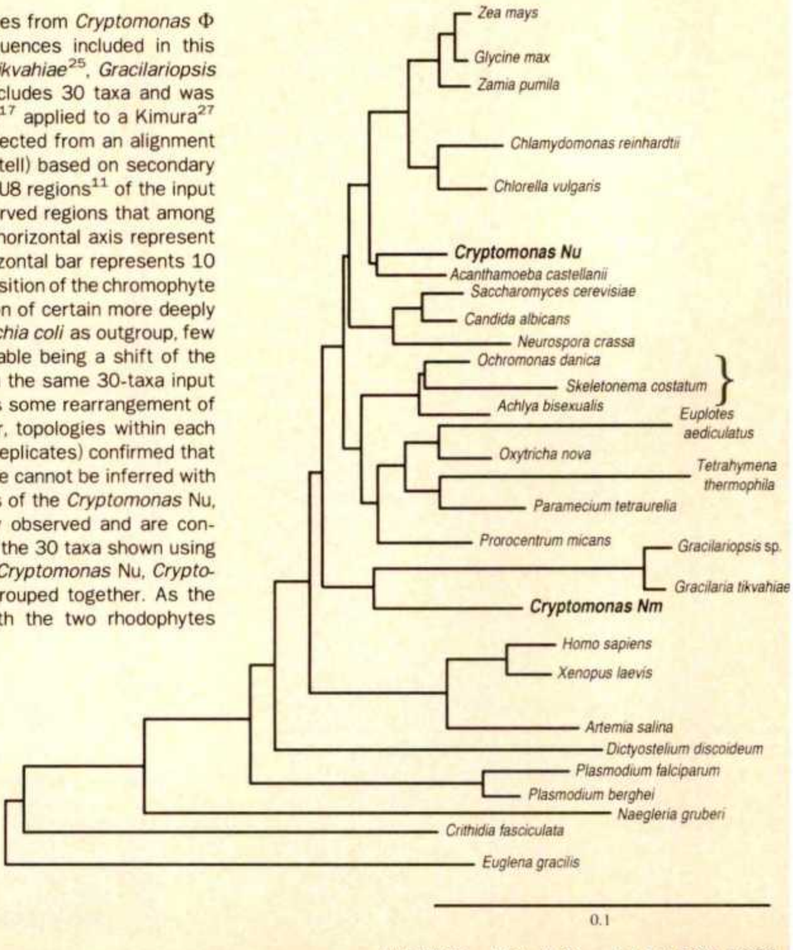


FIG. 3 Detection of transcripts from the Nu and Nm small-subunit rRNA genes. Lane 1, ethidium bromide-stained profile of total cellular RNA (10 μg) from *Cryptomonas* Φ, separated by electrophoresis in a 1.5% agarose–2.2 M formaldehyde gel. Size markers (BRL) are indicated on the left (kb); lane 2, autoradiogram of northern blot hybridized with Nu-specific probe A; lanes 3 and 4, autoradiograms of northern blots hybridized with Nm-specific probes B and C, respectively. For northern hybridization analysis, RNA was transferred to Zeta Probe membranes as described[24], and hybridized with synthetic oligonucleotide probes in 3× SSC, 1% SDS, 10× Denhardt's solution, 20 mM NaH_2PO_4 and 50 μg ml^{-1} sheared single-stranded DNA at 42 °C. Washes were at 20 °C in 2× SSC, 0.1% SDS.

FIG. 4 Phylogenetic analysis of small-subunit rRNA coding sequences from *Cryptomonas* Φ and other eukaryotes. Nuclear-encoded small-subunit rRNA sequences included in this analysis are from ref. 10, with the exception of those from *G. tikvahiae*[25], *Gracilariopsis* sp[25] and *Candida albicans*[26]. This particular phylogenetic tree includes 30 taxa and was constructed using the neighbour-joining method of Saitou and Nei[17] applied to a Kimura[27] dissimilarity matrix. The data set consisted of 1,001 positions selected from an alignment of eukaryotic small-subunit rRNA sequences (supplied by R. R. Gutell) based on secondary structure comparisons; in addition to the universally conserved U1–U8 regions[11] of the input small-subunit rRNAs, the database encompasses other well conserved regions that among eukaryotes can be aligned unambiguously. Branch lengths on the horizontal axis represent the evolutionary distance between the nodes of the tree. The horizontal bar represents 10 changes per 100 nucleotides. The bracket indicates the branching position of the chromophyte algae. When the number of taxa was expanded to 47, with inclusion of certain more deeply branching eukaryotes (*Vairimorpha*, *Giardia*) and employing *Escherichia coli* as outgroup, few changes in overall tree topology were observed, the most noticeable being a shift of the two *Plasmodium* sequences to a position higher in the tree. When the same 30-taxa input data were analysed by a maximum parsimony method[16], there was some rearrangement of major branches relative to the topology shown in Fig. 4; however, topologies within each major group were identical or very similar. Bootstrap analysis (20 replicates) confirmed that the specific branching orders for the major groups higher in the tree cannot be inferred with high confidence. But the relative positions and separate branchings of the *Cryptomonas* Nu, *Cryptomonas* Nm and chromophyte sequences were consistently observed and are considered robust. These branchings are supported by the analysis of the 30 taxa shown using the DNAMove program[16] and starting with an initial tree in which *Cryptomonas* Nu, *Cryptomonas* Nm and the chromophyte *Skeletonema costatum* were grouped together. As the analysis proceeded, *Cryptomonas* Nm affiliated immediately with the two rhodophytes (*Gracilariopsis* sp. and *Gracilaria tikvahiae*), while *Cryptomonas* Nu was progressively displaced away from the chromophytes toward the fungal/chlorophyte/land plant grouping.



shown in Fig. 4, the two form a sister group to chlorophytes and land plants); (3) the *Cryptomonas* Nu sequence does not branch together with members of the chromophyte algae (bracketed arrow, Fig. 4).

Ribosomal RNA sequence comparisons have supported the view that multiple endosymbioses occurred in the formation of land plants and algae[18-20]. It has been argued[3] that only two endosymbiotic events need be invoked to explain the diversity of extant photosynthetic eukaryotes, the first (phagotrophic eukaryotic host plus cyanobacterium) producing those organisms (rhodophytes, chlorophytes and land plants) whose plastids are surrounded by two membranes, the second (a different phagotrophic eukaryote plus primitive eukaryotic alga) giving rise to those algae (including cryptophytes and chromophytes) whose plastids are surrounded by more than two membranes. The latter grouping comprises the proposed kingdom Chromista[21]. Because it is postulated that chromophytes arose from cryptophytes by loss of phycobiliproteins and evolution of chlorophyll c_1 (refs 3, 21), cryptomonads occupy a pivotal position in defining the Chromista. In our analysis, chromophytes form a group separate from *Cryptomonas*, which does not support the concept (based largely on ultrastructural data) of a monophyletic kingdom Chromista[21], in which chromophyte algae are directly derived from a cryptomonad-like ancestor. Our study indicates that cryptophytes, chlorophytes and land plants shared a common ancestor distinct from that which gave rise to the chromophytes. This conclusion is further supported by comparisons of large-subunit[20] and 5S (ref. 5) rRNA sequences and by morphological data[5], all of which suggest that cryptomonads are not particularly closely related to any other algal group.

In conclusion, our results strengthen the hypothesis that the evolutionary formation of cryptomonad algae progressed through two separate stages: a primary endosymbiotic event involving a eukaryotic host and a photosynthetic prokaryote; and a secondary event involving the resulting eukaryotic alga as endosymbiont and a second eukaryote as host. From the small-subunit rRNA sequences encoded by the nuclear genomes of the two eukaryotes contributing to the cryptomonad chimaera, we have been able to discern aspects of the evolutionary history of this complex organism and to assess proposed endosymbiotic events which gave rise to the variety of photosynthetic eukaryotes seen today. Although rRNA sequence comparisons should not be regarded as the only means by which phylogenies can be inferred, a re-evaluation of the concept of the kingdom Chromista[21] as a monophyletic assemblage would be in order, on the basis of our proposal that chromophyte and cryptophyte algae probably arose by different endosymbiotic events. ☐

Received 17 August; accepted 12 December 1990.

1. Gray, M. W. *Trends Genet.* **5,** 294–299 (1989).
2. Whatley, J. M. & Whatley, F. R. *New Phytol.* **87,** 233–247 (1981).
3. Cavalier-Smith, T. *Biol. J. Linn. Soc.* **17,** 289–306 (1982).
4. Gibbs, S. P. *Ann. N.Y. Acad. Sci.* **391,** 193–208 (1981).
5. Ludwig, M. & Gibbs, S. P. *Protoplasma* **127,** 9–20 (1985).
6. Greenwood, A. D., Griffiths, H. B. & Santore, U. J. *Br. Phycol. J.* **12,** 119 (1977).
7. Ludwig, M. & Gibbs, S. P. *Ann. N.Y. Acad. Sci.* **503,** 198–211 (1987).
8. McFadden, G. I. *J. Cell Sci.* **95,** 303–308 (1990).
9. Sogin, M. L. in *PCR Protocols. A Guide to Methods and Applications* (eds Innes, M. A. *et al.*) 307–314 (Academic, San Diego, 1990).
10. Neefs, J.-M., Van de Peer, Y., Henriks, L. & De Wachter, R. *Nucleic Acids Res.* **18** (suppl.), 2237–2247 (1990).
11. Gray, M. W., Sankoff, D. & Cedergren, R. J. *Nucleic Acids Res.* **12,** 5837–5852 (1984).
12. Dahlberg, A. E. *Cell* **57,** 525–529 (1989).
13. Douglas, S. E. *Curr. Genet.* **14,** 591–598 (1988).
14. Schnare, M. N., Heinonen, T. Y. K., Young, P. G. & Gray, M. W. *J. biol. Chem.* **261,** 5187–5193 (1986).
15. Swofford, D. L. PAUP Version 3.0. Illinois Natural History Survey (Champagne, Illinois, 1989).
16. Felsentein, J. PHYLIP Manual Version 3.3 (Herbarium, Univ. California, Berkeley, 1990).
17. Saitou, N. & Nei, M. *Molec. biol. Evol.* **4,** 406–425 (1987).
18. Gunderson, J. H., Elwood, H. J., Ingold, A., Kindle, K. & Sogin, M. L. *Proc. natn. Acad. Sci. U.S.A.* **84,** 5823–5827 (1987).
19. Bhattacharya, D., Elwood, H. J., Goff, L. J. & Sogin, M. L. *J. Phycol.* **26,** 181–186 (1990).
20. Perasso, R., Baroin, A., Qu, L. H., Bachellerie, J. P. & Adoutte, A. *Nature* **339,** 142–144 (1989).
21. Cavalier-Smith, T. *Prog. Phycol. Res.* **4,** 309–347 (1986).
22. Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74,** 5463–5467 (1977).
23. Maxam, A. M. & Gilbert, W. *Meth. Enzym.* **65,** 499–560 (1980).
24. Douglas, S. E. & Durnford, D. G. *Plant molec. Biol.* **13,** 13–20 (1989).
25. Bird, C. J., Rice, E. L., Murphy, C. A., Liu, Q. Y. & Ragan, M. A. *Nucleic Acids Res.* **18,** 4023–4024 (1990).
26. Hendriks, L. *et al. System appl. Microbiol.* **12,** 223–229 (1989).
27. Kimura, M. *J. molec. Evol.* **16,** 111–120 (1980).

ACKNOWLEDGEMENTS. We thank J. Gallant for technical assistance, M. Ragan for critical review, and R. R. Gutell for alignment of eukaryotic 18S rRNA sequences, from which the database used for phylogenetic tree construction was selected. This work was supported by an operating grant from the Medical Research Council of Canada to M.W.G., who is a Fellow in the Evolutionary Biology Program of the Canadian Institute for Advanced Research. This is NRCC publication number 31933. The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession numbers X57162 (nuclear) and X57008 (nucleomorph).

Worldwide migration of amplified insecticide resistance genes in mosquitoes

Michel Raymond*, Amanda Callaghan*†, Philippe Fort‡ & Nicole Pasteur*

* Laboratoire Génétique et Environnement, Institut des Sciences de l'Evolution, URA 327, Case Courrier 64; and ‡ Laboratoire de Biologie Moléculaire, URA 1191, Case Courrier 12; Université Montpellier II, 34095 Montpellier, France

In *Culex pipiens*, overproduction of nonspecific esterases is a common mechanism of resistance to organophosphate insecticides[1,2]. The esterases are attributed to closely linked loci named A and B according to substrate preference[3-6], and overproduction of all esterases B is due to gene amplification[7,8]. Distribution of electrophoretically distinct variants of overproduced esterases A and B is geographically restricted, with the exception of esterases A2 and B2, always found together throughout at least three continents (Fig. 1). To determine whether this situation is due to migration or to a high mutation rate, esterase B structural genes and their flanking regions were compared by sequence and/or restriction fragment length polymorphism analysis. Whereas structural genes were similar, flanking regions of electrophoretically dissimilar esterases B varied considerably. In contrast, flanking sequences of esterases B2 from different geographical locations (Africa, Asia, North America) were identical. These results suggest that amplified esterase B2 genes originated from an initial event that has subsequently spread organophosphate insecticide resistance by migration.

To study the genetic relationship between esterases B1 and B2, the *Hin*dIII-*Bam*HI fragment of the esterase B2 gene was cloned, sequenced and compared with the homologous esterase B1 fragment[9]. The two showed 96% nucleotide sequence homology (not shown), and 97% homology in the predicted amino-acid sequence (Fig. 2). Both esterase B1 and B2 clones were used as probes to construct restriction maps of esterases B from two strains, S-Lab and MSE, which contain a single copy of the gene (each producing an esterase B with a particular electrophoretic mobility different from that of esterase B1 and B2), as well as from mosquitoes with an amplified esterase B1 (Tem-R strain) or an amplified esterase B2 (six strains from different continents: Africa, North America and Asia). Under stringent detection conditions, each map was identical when constructed using either esterase B1 or B2 probes, demonstrating that the probes recognize exactly the same DNA regions. These two results strongly suggest that before amplification, esterase B1 and B2 genes were allelic. In addition, they show that esterase B1 and B2 genes were also alleles of the unamplified esterase B genes observed in S-Lab and MSE. This agrees with results from crossing experiments[10] which established that various unamplified esterases B (different from B1 and B2) are alleles.

A comparison between the different restriction maps (Fig. 3) indicates that the DNA sequences are very similar within the

---

[†] Present address: School of Animal and Microbial Science, University of Reading, Whiteknights, PO Box 228, Reading, RG6 2AJ, UK.

gene among mosquitoes with different esterases. But the number of conserved restriction sites in the sequences flanking the structural genes is relatively small, indicating that the number of mutation events separating any two sequences flanking an esterase B gene is high and that their genetic divergence is probably ancient. In contrast, the whole of the DNA sequences, analysed by restriction mapping, from strains with esterase B2, collected from regions covering three continents, were identical.

The level of polymorphism of the unamplified B locus and its flanking sequences in single mosquitoes was also very high. After only a *Hin*dIII digestion, many alleles were detected (data not shown): at least 15 in a sample of 30 insects from Saint-

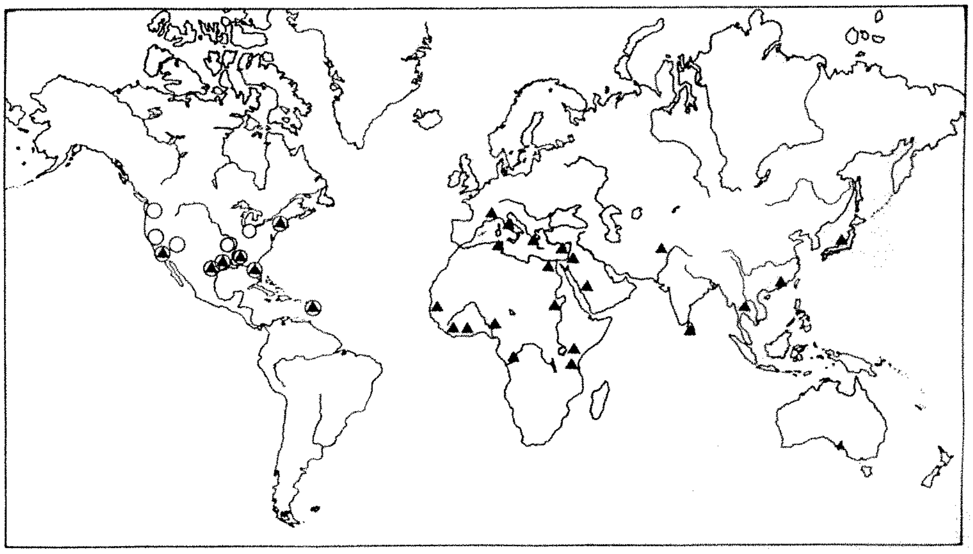


FIG. 1 Known geographic distribution of overproduced esterases B1 (○) and A2–B2 (▲) associated with resistance to organophosphates in mosquitoes of the *Culex pipiens* complex[3,4,11–13,21–25]. The distribution of esterases A2–B2 has increased over the last decade. For example, in California, they appeared before 1984 in an area where until 1980 only esterase B1 had been found[12]; in southern France they were detected in 1986[13] in regions where previously only esterase A1 was present[26], and in Italy they were absent in 1982[27] but present in 1985[3].

```
270 |                 |                 |          *300|               | *               |
B1   KLLTDQDMQDDIFTPFGPTVEPYLTEQCIIPKAPFEMARTAWGDKIDIMIGGTSEEGLLL
B2   ----------------------------M---E-----------R---------------

        |                 |          350|               |               |                 |
B1   LQKIKLHPELLSHPHLFLGNVPPNLKISMEKRIEFAAKLKQRYYPDSIPSMENNLGYVHM
B2   -----------------------------------------------S------------

        |          400|                 |               |               |                 |
B1   MSDRVFWHGLHRTILARAARSRARTFVVYRICLDSEFYNHYRIMMIDPKLRGTAHADELSY
B2   -----------------G-------------------------------------------

  450|                  |                 |                 |
B1   LFSNFTQQVPGKETFEYRGLQTLVDVFSAFVINGD
B2   ---------------------------T-------
```

FIG. 2 Predicted amino-acid sequence similarity (single-letter notation) between esterase B1 and B2 gene products (amino acids 269–482). A fragment of the esterase B2 gene was isolated from a Puc-19 derived *Bam*HI-*Hin*dIII genomic library from the SeLax strain (homozygous for the presence of amplified esterase B2). DNA sequencing used M13 subcloning and dideoxy sequencing[28]. The esterase B2 fragment sequence was compared to that of the esterase B1 *Hin*dIII-*Bam*HI fragment[9]. Conservative amino acids are indicated by an asterisk. The esterase B1 protein sequence is numbered as previously published.

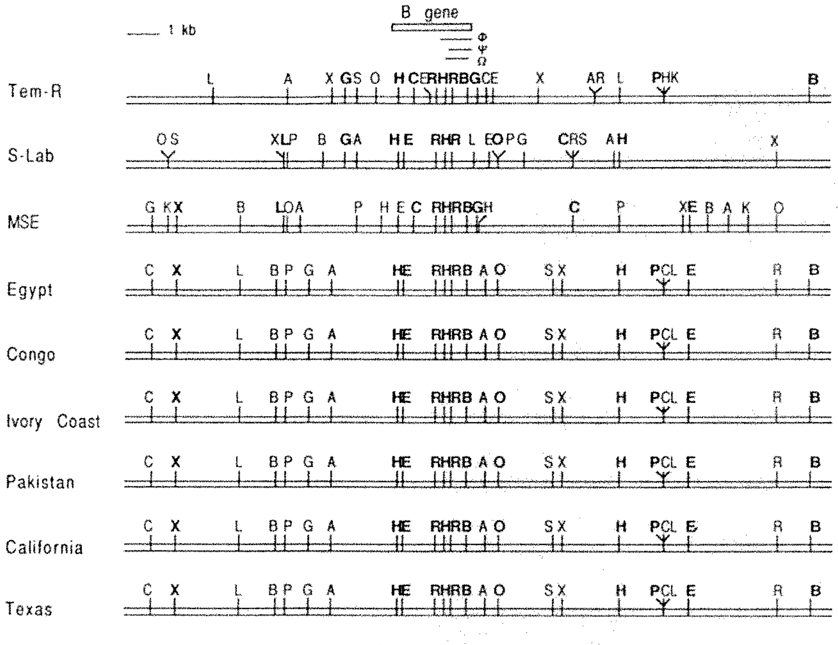

B gene
1 kb
Φ
Ψ
Ω
Tem-R
L A X GS O H CERHRBGCE X AR L PHK B
S-Lab
OS XLP B GA HE RHR L EOPG CRS AH X
MSE
G KX B LOA P H E C RHRBGH C P XE B A K O
Egypt
C X L B P G A HE RHRB A O S X H PCL E R B
Congo
C X L B P G A HE RHRB A O S X H PCL E R B
Ivory Coast
C X L B P G A HE RHRB A O S X H PCL E R B
Pakistan
C X L B P G A HE RHRB A O S X H PCL E R B
California
C X L B P G A HE RHRB A O S X H PCL E R B
Texas
C X L B P G A HE RHRB A O S X H PCL E R B

FIG. 3 Restriction maps of the esterase B genes from organophosphate insecticide-resistant and susceptible adult mosquitoes. The strains used were: Tem-R (ref. 29) from California containing ~250 copies of the esterase B1 gene; S-Lab (ref. 30) a susceptible strain from California; MSE (ref. 31) a low esterase activity strain from southern France; and six strains containing amplified esterase B2 (amplification level ~60-fold), namely Houston[24] and SeLax[12] (Texas and California, North America), Lahore[24] (Pakistan, Asia), and Bouaké[13], Brazaville and Soft[32] (Ivory Coast, Congo and Egypt, Africa).

METHODS. Genomic DNA was extracted from adult mosquitoes using a phenol-chloroform method[8], and digested with one or several restriction enzymes: *Acc*I (A), *Bam*HI (B), *Bcl*I (C), *Bgl*II (G), *Hin*dIII (H), *Eco*RI (E), *Eco*RV (R), *Kpn*I (K), *Pst*I (P), *Sac*I (S), *Sal*I (L), *Xba*I (X) and *Xho*I (O). The resulting DNA fragments were blotted onto nylon filters (Hybond[N]) after the method of Southern[33], and visualized by hybridization to the ^{32}P-labelled 0.7- or 1.3-kilobase (kb) complementary DNA clones of the esterase B1 gene[7,9], or to the 0.7-kb genomic esterase B2 clone. The filters were washed stringently to ensure that only closely related DNA fragments were being studied. Above the restriction maps are indicated the localizations of the esterase B gene (shaded box), of the 1.3-kb (Φ) and 0.7-kb (Ψ) cDNA esterase B1 probes, and of the 0.7-kb (Ω) esterase B2 genomic probe.

Martin-de-Londres (France), and 13 (all different from the first 15) in a sample of 28 mosquitoes from Sintra (Portugal). None of these were unamplified B1 or B2 alleles.

These observations suggest that all amplified esterase B2 genes and flanking sequences have arisen from a single initial event and support the hypothesis that the worldwide presence of esterase B2 amplification is due to recent migration. This conclusion agrees well with the strong linkage disequilibrium, also observed worldwide, between esterases B2 and A2, which can best be explained by a rapid migration of an initial A2-B2 gene association. The place and time of the occurrence of the original amplification event cannot be traced, but Africa or Asia is most likely[4,11]. The recent history of the sudden appearance of esterases A2-B2 is well documented for California, Italy and France[3,12,13] and a migration between continents is not unlikely, as live larvae and adults of the *C. pipiens* complex are often found on international flights and boats[14-16].

The rapid spread of B2 esterase gene amplification since its probable occurrence in the 1960s (when the intensive use of organophosphate insecticides began) can be compared with that of the *Drosophila melanogaster* alcohol dehydrogenase *Fast* allele associated with high alcohol dehydrogenase activity. There is less restriction polymorphism among *Fast* than *Slow* alleles[17-20]. This has been interpreted to reflect a recent origin of the *Fast* allele, perhaps within the past 2,000 years[17]. Its presence, mainly in temperate areas, may result from an extensive migration and an unknown selective factor.

Resistance genes (or genetic factors such as gene amplification) may arise in a population under insecticide control by mutation or by migration. In *C. pipiens*, very few factors conferring significant organophosphate resistance have been selected during the past 30 years, indicating that the genetic possibilities are limited. As each has a relatively low probability of occurrence, the evolution of organophosphate resistance in a treated population is more likely to be determined by the extent of migration rather than by the rate of mutation. □

Received 16 August; accepted 21 December 1990.

1. Fournier, D. *et al. Pest. Biochem. Physiol.* **27,** 211–217 (1987).
2. Mouchès, C. *et al. Proc. natn. Acad. Sci. U.S.A.* **84,** 2113–2116 (1987).
3. Callaghan, A. thesis, Univ. of London (1989).
4. Villani, F., White, G. B., Curtis, C. F. & Miles, S. J. *Bull. ent. Res.* **73,** 153–170 (1983).
5. Pasteur, N., Iseki, A. & Georghiou, G. P. *Biochem. Genet.* **19,** 909–919 (1981).
6. Wirth, M. C., Marquine, M., Georghiou, G. P. & Pasteur, N. *J. med. Entomol.* **27,** 202–206 (1990).
7. Mouchès, C. *et al. Science* **233,** 778–780 (1986).
8. Raymond, M. *et al. Biochem. Genet.* **27,** 417–423 (1989).
9. Mouchès, C. *et al. Proc. nantn. Acad. Sci. U.S.A.* **87,** 2574–2578 (1990).
10. de Stordeur, E. *Biochem. Genet.* **14,** 481–493 (1976).
11. Curtis, C. F. & Pasteur, N. *Bull. ent. Res.* **71,** 153–161 (1981).
12. Raymond, M. *et al. J. med. Ent.* **24,** 24–27 (1987).
13. Magnin, M. thesis, Univ. Paris VI (1986).
14. Andreadis, T. G. *J. Am. Mosq. Control Assoc.* **4,** 256–260 (1988).
15. Asahina, S. *Jap. J. Med. Sci. Biol.* **23,** 255–258 (1970).
16. Highton, R. B. & Van Someren, E. C. C. *Bull. WHO* **42,** 334–335 (1970).
17. Aquadro, C. F., Desse, S. F., Bland, M. M., Langley, C. H. & Laurie-Ahlberg, C. C. *Genetics* **114,** 1165–1190 (1986).
18. Kreitman, M. & Aguadé, M. *Proc. natn. Acad. Sci. U.S.A.* **83,** 3562–3566 (1986).
19. Aguadé, M. *Genetics* **119,** 135–140 (1988).
20. Simmons, G. M., Kreitman, M., Quattlebaum, W. F. & Miyashita, N. *Evolution* **43,** 393–409 (1989).
21. Pasteur, N. & Georghiou, G. P. *J. Econ. Entomol.* **82,** 347–353 (1989).
22. Magnin, M., Marboutin, E. & Pasteur N. *J. med. Ent.* **25,** 99–104 (1988).
23. Hemingway, J., Callaghan, A. & Amin, A. *Med. Vet. Ent.* **3,** 445–446 (1989).
24. Beyssat-Arnaouty, V., Mouchès, C., Georghiou, G. P. & Pasteur, N. *J. Am. Mosq. Control Assoc.* **5,** 196–200 (1989).
25. Urbanelli, S., Bullini, L. & Villani, F. *Bull. ent. Res.* **75,** 291–304 (1985).
26. Pasteur, N., Sinègre, G. & Gabinaud, A. *Biochem. Genet.* **19,** 499–508 (1981).
27. Villani, F. & Hemingway, J. *J. Pest. Biochem. Physiol.* **27,** 218–228 (1987).
28. Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74,** 5463–5467 (1977).
29. Georghiou, G. P. & Pasteur, N. *J. Econ. Ent.* **73,** 489–492 (1980).
30. Georghiou, G. P., Metcalf, R. L. & Glidden, F. E. *Bull. WHO* **35,** 691–708 (1966).
31. Raymond, M. *et al. C.r. Acad. Sci. Paris* **300,** 509–512 (1985).
32. Beyssat-Arnaouty, V. thesis, Univ. Montpellier II (1989).
33. Southern, E. M. *J. molec. Biol.* **98,** 503 (1975).

ACKNOWLEDGEMENTS. We thank M. Marquine for technical assistance, G. P. Georghiou and I. Olivieri for providing strains, G.P.G. for allowing us to use his unpublished data, V. Beyssat, P. Borsa, V. Perrot, E. Poulin, L. Pouyaud, and M. A. Réglade for help in restriction mapping, and P. Jarne, C. Malcolm and Y. Michalakis for helpful discussions. A.C. was the recipient of a Royal Society/CNRS fellowship. This work was supported by PIREN/CNRS and MRT.

Action potentials must admit calcium to evoke transmitter release

Rosel M. Mulkey & Robert S. Zucker

Division of Neurobiology, Department of Molecular and Cell Biology,
University of California, Berkeley, California 94720, USA

THERE are two hypotheses to explain how neurons release transmitter. The calcium hypothesis proposes that membrane depolarization is necessary only for opening calcium channels and increasing internal calcium concentration ($[\mathrm{Ca}^{2+}]_i$) near membrane transmitter-release sites[1-3]. These calcium ions trigger a transient release of neurotransmitter[4,5]. The calcium-voltage hypothesis postulates that voltage induces a conformational change in a membrane protein rendering it sensitive to calcium such that, in the presence of high $[\mathrm{Ca}^{2+}]_i$, depolarization directly triggers transmitter release[6-9]. Here we report that when calcium influx is blocked by cobalt or manganese ions in a calcium-free Ringer, as measured with Fura-2, and $[\mathrm{Ca}^{2+}]_i$ is elevated by liberation from a caged calcium compound, transmitter release at the crayfish neuromuscular junction is unaffected by presynaptic action potentials. These results support the calcium hypothesis.

To distinguish between the hypotheses we had to independently increase $[\mathrm{Ca}^{2+}]_i$ in terminals and depolarize the membrane without allowing calcium influx. New photolabile 'caged' calcium compounds decrease their affinity for calcium on exposure to ultraviolet light and permit the elevation of $[\mathrm{Ca}^{2+}]_i$ in presynaptic terminals without depolarizing the membrane. A direct effect of voltage on transmitter release at crayfish neuromuscular junctions was inferred recently[10] from postsynaptic responses to presynaptic action potentials in low-calcium solutions containing calcium-channel blockers, following elevation of presynaptic $[\mathrm{Ca}^{2+}]_i$ with the photosensitive calcium chelator nitr-5 or the mitochondrial poison carbonyl cyanide *m*-chlorophenylhydrazine. Upon testing these low-calcium solutions used in ref. 10 by using the calcium indicator dye Fura-2, we found that calcium influx was not fully blocked, and transmitter release was clearly evoked by presynaptic action potentials at high stimulus frequencies. Therefore, the conclusion that voltage has a direct effect on transmitter release is invalid, because membrane depolarization activated calcium influx. We reexamined the calcium-voltage hypothesis at the crayfish neuromuscular junction using the photolabile calcium chelator DM-nitrophen and tested solutions containing calcium-channel blockers using the calcium indicator Fura-2 to be certain calcium influx was abolished during membrane depolarization.

The fluorescent dye Fura-2 was iontophoresed into the excitatory motor axon of the first walking leg of the crayfish, *Procambarus clarkii*, and intracellular $[\mathrm{Ca}^{2+}]_i$ in presynaptic boutons was determined ratiometrically from signals obtained with 350 and 385 nm illumination. Excitatory postsynaptic potentials (e.p.s.ps) were recorded simultaneously in muscle fibres where the imaged terminals synapsed or in adjacent fibres. An extracellular electrode on an axonal branch recorded nerve field potentials to confirm action potential invasion of terminals. A nerve field potential was also sometimes observed in the postsynaptic recording. We used the same stimulation paradigm employed in ref. 10 (twin pulses, 20 ms apart delivered at 10 Hz) as well as high frequency stimulation at 100 Hz.

Figure 1*a* exemplifies experiments in which $[\mathrm{Ca}^{2+}]_i$ rose by 87 ± 17 nM (mean ± s.d., 21 terminals in 10 preparations designated $N = 21, 10$) during a 2-min twin-pulse stimulation in 'calcium-free Ringer's solution' containing 12.5 mM magnesium. Even though there was a small calcium influx at this frequency, transmitter release was undetectable, owing to the highly nonlinear relationship between calcium and release[11] (Fig. 1*b* and ref. 10). At 100 Hz for 2 min, $[Ca^{2+}]_i$ increased from a resting value of 104 ± 45 nM to approximately 923 ± 313 nM ($N = 9, 5$) (Fig. 1*c*). This large increase in $[Ca^{2+}]_i$ facilitated transmitter release sufficiently that it was now easily detectable (Fig. 1*d*). When such experiments were done using a 'manganese Ringer's' containing 0.2 mM calcium, 2 mM manganese, and 12.5 mM magnesium, a large increase in $[Ca^{2+}]_i$ was seen at frequencies

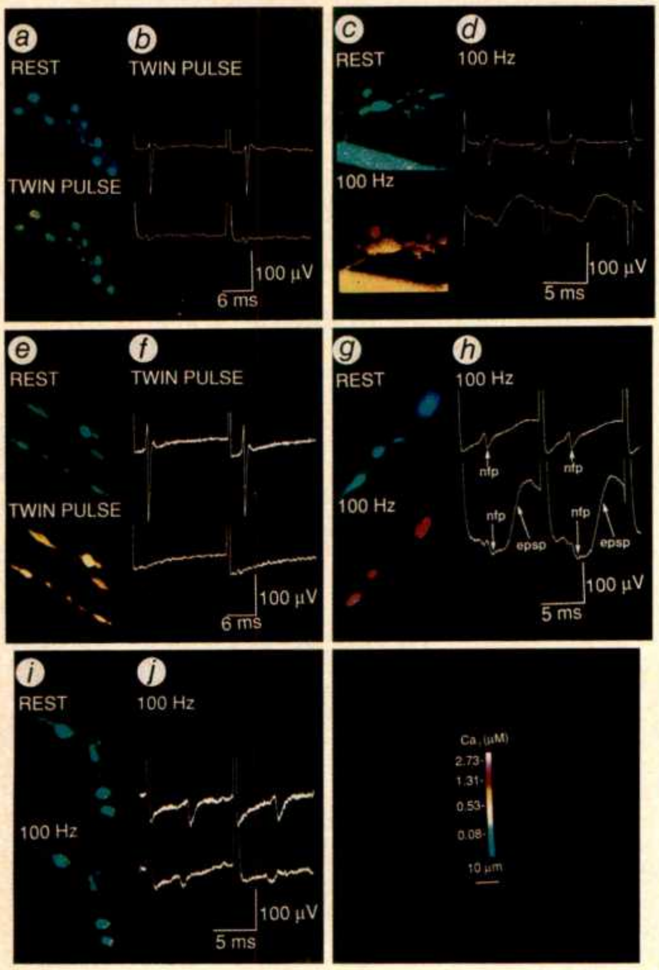


FIG. 1 Measurements of intracellular calcium and evoked transmitter release during presynaptic stimulation in 'calcium-free Ringer's' containing 12.5 mM Mg (*a–d*), 'manganese Ringer's' containing 0.2 mM Ca, 2 mM Mn, and 12.5 mM Mg (*e–h*), or 'cobalt Ringer's' containing 13.5 mM Co and 30 mM Mg (*i, j*). Each panel is from a different preparation. *a, e*, $[Ca^{2+}]_i$ measured at rest and during twin-pulse stimulation and *c, g, i*, 100 Hz stimulation. *b, f*, Averaged e.p.s.ps (bottom trace) and nerve field potentials (n.f.ps, top trace) recorded during twin-pulse stimulation and *d, h, j*, during 100 Hz stimulation. Neither calcium-free magnesium Ringer's nor manganese Ringer's with some calcium added prevented calcium influx and transmitter release; the cobalt Ringer's with no added calcium prevented both.

METHODS. The excitatory axon of the first walking leg of the crayfish (*Procambarus clarkii*), was injected with Fura-2 as described in ref. 13. Individual presynaptic terminals were imaged using a ×40 Zeiss water immersion lens and Nikon Optiphot microscope equipped with a Dage silicon intensified target camera (Model 66). $[Ca^{2+}]_i$ was calculated from 'ratioed' fluorescence images with excitation at 350 and 385 nm (ref. 13). The excitatory axon was stimulated with a suction electrode placed on the nerve dissected from the meropodite. The e.p.s.ps were recorded with an intracellular electrode containing 3 M KCl (10 MΩ) placed in a muscle fibre where imaged terminals synapsed. An extracellular electrode (25–50 µm diameter), placed on an axonal branch on the surface of the opener muscle, recorded the nerve field-potential at its time of arrival in this particular branch. The e.p.s.ps and motor nerve field-potentials were averaged on a digital oscilloscope (Nicolet 4094, Madison). Normal Ringer's contained (in mM) 195 NaCl, 13.5 CaCl, 5.4 KCl, 2.6 MgCl, and 10 sodium–HEPES at pH 7.4. Temperature was maintained at 18 °C. A gravity perfusion system with inflow above the opener muscle provided continuous perfusion with the appropriate saline solution. To ensure complete removal of calcium from the bath, the objective was lifted out of the bath and the muscle surface washed vigorously with a syringe having a 1-mm-diameter plastic tube attached to the tip. A perfusion time of 5–10 min was needed to eliminate calcium influx during a high-frequency train. We found that a manganese Ringer's with no added calcium was equivalent to the cobalt Ringer's in preventing calcium influx. When both calcium and manganese were present in the Ringer, a rise in $[Ca^{2+}]_i$ was measurable for only 20 s at 100 Hz. Manganese ions appear to pass through calcium channels in presynaptic terminals[14] and then bind to Fura-2 and cause quenching of its fluorescence[15-17]. From the intensity of Fura-2 fluorescence, we estimate its concentration at 100–150 µM in boutons. Complete quenching suggests that about 100 µM manganese accumulates during a 20 s, 100 Hz train. This may be compared to a rise in free $[Ca^{2+}]_i$ of over 5 µM at 100 Hz in normal Ringer's[13]. About 99% of entering calcium is bound to cytoplasmic buffers[18], so the influx of manganese through calcium channels is less than 20% that of calcium. We preferred the cobalt Ringer's because quenching of the Fura-2 signal took longer (40–60 s), suggesting that cobalt is less permeable than manganese through calcium channels.

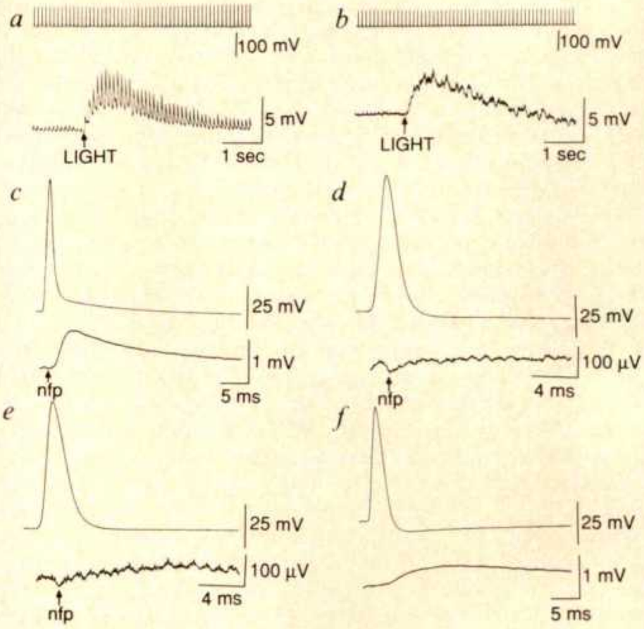


FIG. 2 Effect of action potentials on transmitter release in the absence of calcium entry. In each panel, the upper trace shows presynaptic action potentials stimulated at 10 Hz and the lower trace shows postsynaptic potentials. *a*, control experiment on a preparation injected presynaptically with DM-nitrophen and bathed in normal calcium solution. Photolysis with a 10-s-exposure to UV light beginning at the arrow elevated presynaptic $[Ca^{2+}]_i$ and triggered transmitter release, seen as a depolarizing shift in the postsynaptic baseline. Spike-evoked e.p.s.ps were also strongly facilitated. The gradual decline of the response probably reflects active extrusion of the photo-released calcium from the terminals and depletion of releasable transmitter quanta. The remaining five panels are from a different preparation showing that action potentials evoked no transmitter release in cobalt Ringer which effectively prevents calcium influx. *b*, Presynaptically injected nitrophen was photolysed to trigger transmitter release directly. Both before and during the light exposure, presynaptic spikes evoked no release in cobalt Ringer's. *c–f*, Averages of 50 responses to presynaptic stimulation in the experiment of panel *b*. *c*, Normal e.p.s.ps evoked by action potentials in normal Ringer's. A small field potential reflecting invasion of the presynaptic terminals by action potentials preceded the e.p.s.p. *d*, E.p.s.ps blocked by cobalt Ringer's but nerve terminal potential remaining, indicating that presynaptic terminals were still invaded. *e*, First 50 responses during photolysis of the nitrophen. The nerve terminal potential was still evident, and the increased noise reflected an acceleration in miniature e.p.s.p. frequency following elevation of $[Ca^{2+}]_i$. Nevertheless, action potentials evoked no transmitter release in the absence of calcium influx. When normal extracellular calcium was restored (*f*), action potentials again admitted calcium presynaptically, and e.p.s.ps returned.

METHODS. Bevelled microelectrodes were filled with 60 mM DM-nitrophen, 18 or 36 mM $CaCl_2$, and 10 mM sulphorhodamine B (Molecular Probes) in 0.5 M potassium–HEPES, pH 7.3. The exciter axon was impaled near the Y-branch on the muscle surface and filled by pressure injection as described in ref. 19. By including 10 mM sulphorhodamine B (Molecular Probes) in the electrode, we could monitor filling the terminals without photolysing the DM-nitrophen, which reached a final concentration of about 3 mM. Light from a 100 W mercury epifluorescence illuminator was attenuated with a 25% transmission N.D. filter and focused onto terminals through a ×10, 0.25 n.a. objective to photolyse about 20% of the nitrophen within 1 s, raising $[Ca^{2+}]_i$ from 0.1–0.5 µM to several micromolar. The exact levels of $[Ca^{2+}]_i$ depend on concentrations of magnesium, ATP, DM-nitrophen, and native calcium buffer in nerve terminals[19], and are presently undetermined. When nitrophen was injected without calcium loading in calcium-free media, photolysis evoked little or no transmitter release, indicating that release of magnesium from nitrophen does not trigger the responses. The injection electrode recorded the presynaptic action potential and a postsynaptic electrode was placed in a muscle fibre onto which filled terminals synapsed and from which a motor nerve field-potential could be recorded. Miniature e.p.s.p. frequency was estimated from the postsynaptic depolarization as described in ref. 5. A calcium-activated potassium current ($I_{K(Ca)}$) has been reported in crayfish presynaptic terminals[20]. To prevent shunting of action potentials following $[Ca^{2+}]_i$ elevation by nitrophen photolysis, tetraethylammonium was added to cobalt Ringer's at 2 mM, a dose which is effective in blocking $I_{K(Ca)}$ (M. Wojtowicz, personal communication).

of 100 Hz, from 69 ± 25 nM at rest to 900 ± 606 nM ($N = 11, 5$) in 20 s (Fig. 1*g*), and synaptic potentials were also observed (Fig. 1*h*). Twin pulse stimulation for 90 s in manganese Ringer's produced a 189 ± 55 nM ($N = 8, 4$) increase in $[Ca^{2+}]_i$ (Fig. 1*e*) but, as in ref. 10, no detectable evoked transmitter release (Fig. 1*f*).

Since these calcium-free and manganese Ringer's permit calcium influx during membrane depolarization, this would cause evoked transmitter release which can be facilitated to detectable levels by elevating presynaptic $[Ca^{2+}]_i$, either through high frequency stimulation or the use of photolabile calcium chelators or mitochondrial poisons[10]. Therefore, such release does not support the calcium-voltage hypothesis. We tested this hypothesis by using the photolabile calcium chelator DM-nitrophen and solutions which more effectively prevent calcium entry. We chose DM-nitrophen, instead of nitr-5, because it binds calcium with a higher affinity before photolysis and a lower affinity after light exposure[12], providing bigger changes in $[Ca^{2+}]_i$. We found that a 'cobalt Ringer's' containing 13.5 mM cobalt, 30 mM magnesium, and no added calcium, permits only minimal calcium entry during action potentials. The $[Ca^{2+}]_i$ rose by only 64 ± 19 nM ($N = 11, 5$) during a 20-s 100-Hz stimulation, and no transmitter release was observed (Fig. 1*i*, *j*). The small rise in $[Ca^{2+}]_i$ and the presence of nerve field potentials indicate that action potentials were invading the nerve terminals but that calcium influx was insufficient to evoke transmitter release. A zero-calcium saline containing 2 mM EGTA was even more effective in reducing calcium influx but EGTA often led to presynaptic depolarization and failure of the action potential.

With a solution that effectively prevents calcium influx, it was possible to test the calcium-voltage hypothesis. In normal Ringer's, DM-nitrophen photolysis strongly increased transmitter release, expressed as the frequency of appearance of miniature e.p.s.ps, by 1,700-22,500% ($N = 12$); evoked e.p.s.ps were facilitated by 173-2,188%. These effects are much more powerful than those seen using nitr-5 (ref. 10).

Figure 2*b*-*f* illustrates one of six experiments to test for an effect of voltage on transmitter release without calcium influx. Initially, action potentials elicited large e.p.s.ps in normal Ringer's, in addition to a small nerve terminal potential recorded by the muscle electrode (Fig. 2*c*). After rinsing in cobalt Ringer's for 10 min, 10-Hz stimulation failed to evoke transmitter release, but the muscle electrode still recorded a nerve terminal potential (Fig. 2*d*). Photolysis of nitrophen triggered intense transmitter release, with miniature frequency rising from about 1 Hz to 4,500 Hz (800-10,700 Hz, $N = 6$) (Fig. 2*b*). Nevertheless, action potentials still failed to activate transmitter release (Fig. 2*e*). On return to normal Ringer's the e.p.s.p. reappeared in 3-4 min, smaller in amplitude, but broader than the control synaptic potential (Fig. 2*f*), due to the residual effects of TEA and incomplete exchange of solutions.

These and previous[10] results lead us to the following conclusions about synaptic transmission at the crayfish neuromuscular junction: (1) saline solutions containing magnesium or manganese and low calcium permit calcium influx and presynaptic accumulation during action potentials and produce evoked release at high stimulus frequency; this transmitter release is potentiated by elevation of resting $[Ca^{2+}]_i$; (2) solutions containing cobalt (or manganese or EGTA) and no added calcium allow minimal calcium influx and as a result do not produce transmitter release to action potentials; (3) elevation of $[Ca^{2+}]_i$ in crayfish presynaptic boutons through photolysis of DM-nitrophen causes a large facilitation of evoked transmitter release and increases miniature e.p.s.p. frequency in a normal calcium medium; (4) in solutions which effectively block calcium influx during action potentials, elevation of $[Ca^{2+}]_i$ through photolytic release of 'caged' calcium strongly activates transmitter release, but the depolarization of an action potential produces no additional release; (5) the normal spike-evoked secretion of neurotransmitter is not affected directly by presynaptic voltage but is triggered exclusively by an increase in $[Ca^{2+}]_i$ near transmitter release sites. □

Received 25 September; accepted 13 December 1990.

1. Zucker, R. S. & Landó, L. *Science* **231,** 574-579 (1986).
2. Zucker, R. S. & Haydon, P. G. *Nature* **335,** 360-362 (1988).
3. Zucker, R. S., Landò, L. & Fogelson, A. L. *J. Physiol., Paris* **81,** 237-245 (1986).
4. Katz, B. *The Release of Neural Transmitter Substances.* (Thomas, Springfield, 1969).
5. Miledi, R. *Proc. R. Soc.* B**183,** 421-425 (1973).
6. Llinás, R., Steinberg, I. Z. & Walton, K. *Biophys. J.* **33,** 323-352 (1981).
7. Dudel, J., Parnas, I. & Parnas, H. *Pflügers Arch.* **399,** 1-10 (1983).
8. Parnas, I., Parnas, H. & Dudel, J. *Pflügers Arch.* **406,** 121-130 (1986).
9. Parnas, I., Parnas, H. & Dudel, J. *Pflügers Arch.* **406,** 131-137 (1986).
10. Hochner, B., Parnas, H. & Parnas, I. *Nature* **342,** 433-435 (1989).
11. Zucker, R. S. *A. Rev. Neurosci.* **12,** 13-31 (1989).
12. Kaplan, J. H. & Ellis-Davies, G. C. R. *Proc. natn. Acad. Sci. U.S.A.* **85,** 6571-6575 (1988).
13. Delaney, K. R., Zucker, R. S. & Tank, D. W. *J. Neurosci.* **9,** 3558-3567 (1989).
14. Narita, K., Kawasaki, F. & Kita, H. *Brain Res.* **510,** 289-295 (1990).
15. Hallam, T. J., Jacob, R. & Merritt, J. E. *Biochem. J.* **255,** 179-184 (1988).
16. Foder, B., Scharff, O. & Thastrup, O. *Cell Calcium* **10,** 477-490 (1989).
17. Grynkiewicz, G., Poenie, M. & Tsien, R. Y. *J. biol. Chem.* **260,** 3440-3450 (1985).
18. Fogelson, A. L. & Zucker, R. S. *Biophys. J.* **48,** 1003-1017 (1985).
19. Delaney, K. R. & Zucker, R. S. *J. Physiol.* **426,** 473-498 (1990).
20. Bittner, G. D., *J. Neurobiol.* **20,** 386-408 (1988).

ACKNOWLEDGEMENTS. We thank J. Kaplan and G. Ellis-Davies for DM-nitrophen, R. Y. Tsien for help in assembling the ratiometric imaging microscope, R. E. English for technical assistance, and K. Delaney for discussion. This research was supported by the NIH (R.S.Z. and R.M.).

Nature of the motor element in electrokinetic shape changes of cochlear outer hair cells

Peter Dallos, Burt N. Evans & Richard Hallworth

Auditory Physiology Laboratory (The Hugh Knowles Center) and
Department of Neurobiology and Physiology, Northwestern University,
Evanston, Illinois 60208, USA

IT is the prevailing notion that cochlear outer hair cells function as mechanical effectors as well as sensory receptors[1-3]. Electrically induced changes in the shape of mammalian outer hair cells[4,5], studied *in vitro*, are commonly assumed to represent an aspect of their effector process that may occur *in vivo*. The nature of the motile process is obscure, even though none of the established cellular motors can be involved[6]. Although it is known that the motile response is under voltage control[7], it is uncertain whether the stimulus is a drop in the voltage along the long axis of the cell or variation in the transmembrane potential. We have now performed experiments with cells partitioned in differing degrees between two chambers. Applied voltage stimulates the cell membrane segments in opposite polarity to an amount dependent on the partitioning. The findings show, in accordance with previous suggestions[6,8], that the driving stimulus is a local transmembrane voltage drop and that the cellular motor consists of many independent elements, distributed along the cell membrane and its associated cortical structures. We further show that the primary action of the motor elements is along the longitudinal dimension of the cell without necessarily involving changes in intracellular hydrostatic pressure. This establishes the outer hair cell motor as unique among mechanisms that control cell shape[9].

Mammalian outer hair cells are slender cylindrical structures of fairly uniform diameter (~8-10 μm) and their length ranges from ~20-30 μm in the high-frequency cochlear base, to about 80-100 μm in the low-frequency apex. These cells are distinguished by the presence of several layers of subsurface cisterns[10,11] and an elaborate network of cortical cytoskeletal elements between the cisterns and the plasma membrane[12-14]. Outer hair cells possess primarily efferent innervation with only a rudimentary afferent endowment[15,16] that constitutes 5-10% of the cochlear outflow[17]. Yet, interference with outer hair cell function produces profound changes in afferent neural and behavioural responses[18,19], implying a mediating effect on cochlear function. The electromotile mechanism[4] is conceivably the vehicle for this mediation. Passive mechanisms of electrophoresis or electro-osmosis driven by longitudinal voltage drops along the cell have been proposed[20-22]. An alternative suggestion is that transmembrane voltage drop drives the longitudinal motility through a change in circumference, coupled to longitudinal length change by the intermediate step of changing internal hydrostatic pressure[6,8].

In our experiments, isolated outer hair cells are inserted part-way into a pipette, designated the microchamber (Fig. 1), that is loosely patterned after the suction pipette used for examining retinal rods[23]. The imposed voltage (V) drops on the series impedances of the included and extruded membrane segments, which form a voltage divider (Fig. 2*a*). Whereas current flow in the cytoplasm is unidirectional, it is inward through one membrane segment and outward through the other. Consequently, the voltage drops on the included and excluded membrane segments are in opposite polarity and have approximate magnitudes of pV and $(1-p)V$, where p is the fraction of the cell length extruded from the chamber. The key observation is shown in Fig. 3*a*. The two poles of the cell move in opposite phase; when the basal portion contracts, the apical portion elongates and vice versa. Displacements increase cumulatively from zero at the attachment of the cell to the microchamber, to maximum at the two cellular poles (Fig. 2*b*). These observations are independent of the waveform of the stimulus; here we demonstrate the response to a sinusoidal voltage command. Because the longitudinal intracellular voltage drop is unidirectional, whereas the included and extruded cell segments change their lengths in opposite phase, we conclude that the motor is driven by local membrane potential gradient.

A membrane-associated motor could work by one of two conceptually distinct mechanisms. The elementary motors could elongate or contract along the cell axis, thereby producing length changes directly. Alternatively the elementary motors could deform circumferentially[6]. This would produce increments (for radial contraction) or decrements (for radial expansion) in the cell's internal hydrostatic pressure. So that constant cell volume may be maintained, the cell would elongate or shorten. Support for this possibility comes from observations showing that radial contraction–expansion cycles accompany longitudinal motility (ref. 8 and our own measurements). The microchamber method allows the direct testing of the two hypotheses. The number of motor elements in the extruded segment is proportional to p, whereas that in the included segment to $(1-p)$. Thus, if the longitudinal mechanism were correct, the length change of either extruded or included segments would be dependent on the product of the effective voltage and number of motor elements, $p(1-p)$, but would be in the opposite direction. This function is maximum when the cell is half inserted into the microchamber, $p = 0.5$ (Fig. 3*b*). But if longitudinal motility relied on internal pressure change driven by circumferential motors, the motile response should be very small and the two ends ought to move in phase. This is expected because internal pressure changes, if they were produced in the two cell segments, would be equal and of opposite sign. Moreover, any net residual pressure change

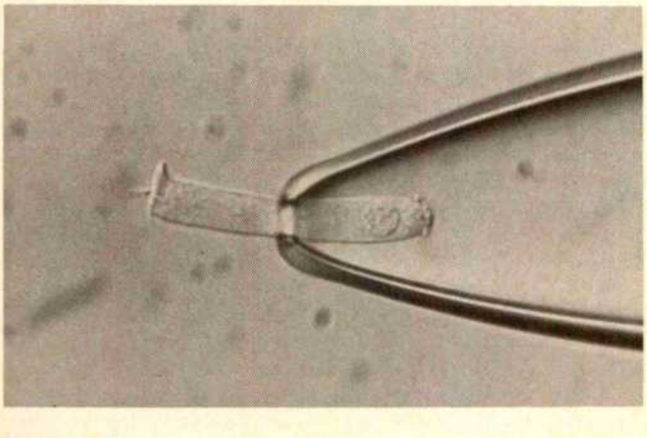


FIG. 1 Photographic view of the microchamber with outer hair cell partially inserted.

METHODS. Outer hair cells are isolated from guinea-pig cochleas by enzymatic digestion (medium is as in Leibovitz's L-15 but omitting everything except monovalent ion salts) and gentle trituration[11]. The solution is supplemented by 15 mM HEPES buffer and 0.1% papain (Sigma) and adjusted to pH 6.9–7.0, osmolarity 310 mosmol. The microchamber is fabricated from borosilicate thin-wall glass tubing (2-mm outer diameter) pulled to a rapidly tapering tip of 10 μm. The tip is then heat polished to a final inner diameter of 8 μm. The final taper of the pipette is sufficiently rapid so that inserted cells are free of the chamber wall. The pipette is bent at 45 ° approximately 5 mm from the tip and coated with a silane compound to prevent adherence between chamber and cell. The microchamber is mounted in an experimental bath on the stage of a Zeiss inverted microscope.

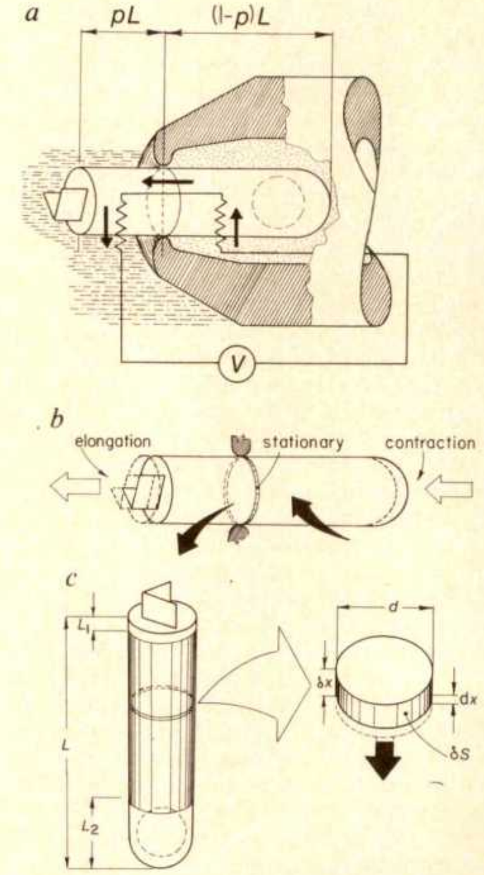


FIG. 2 *a*, Schematic view of the microchamber with a partially inserted cell. The voltage applied between the bath and the electrolyte filling the microchamber (the same solution in all these experiments: Leibovitz's L-15 (Gibco) buffered with 15 mM HEPES and adjusted to pH 7.35, 310 mOsmol) is V. *b*, Schematic of currents (black arrows) and resulting motions of the cell's two poles. When one end shortens, the other extends. This appears to the observer as if both ends moved in the same direction, that is, as if the cell moved in and out of the microchamber. But by closely observing cytoplasmic landmarks we satisfied ourselves that there is no motion at the narrow ring where the cell is attached to the microchamber. Noting the movement of various cellular features, it is clear that movement amplitude cumulatively increases away from the attachment site. *c*, Elementary circumferential ring, assumed to be the 'motor unit', of surface area δS and length δx. A voltage drop δV across the surface is presumed to result in longitudinal contraction (for depolarization) or extension (for hyperpolarization) of magnitude dx. Total motion is assumed to be the sum of elementary motions: $x = \Sigma\, dx$[6,25]. The motor elements are taken to be coincident with the cell cortex that relates to subsurface cisterns and cortical lattice ($L - L_1 - L_2$). The region L_1 corresponds to the cuticular plate, whereas L_2 to the nuclear and synaptic region.

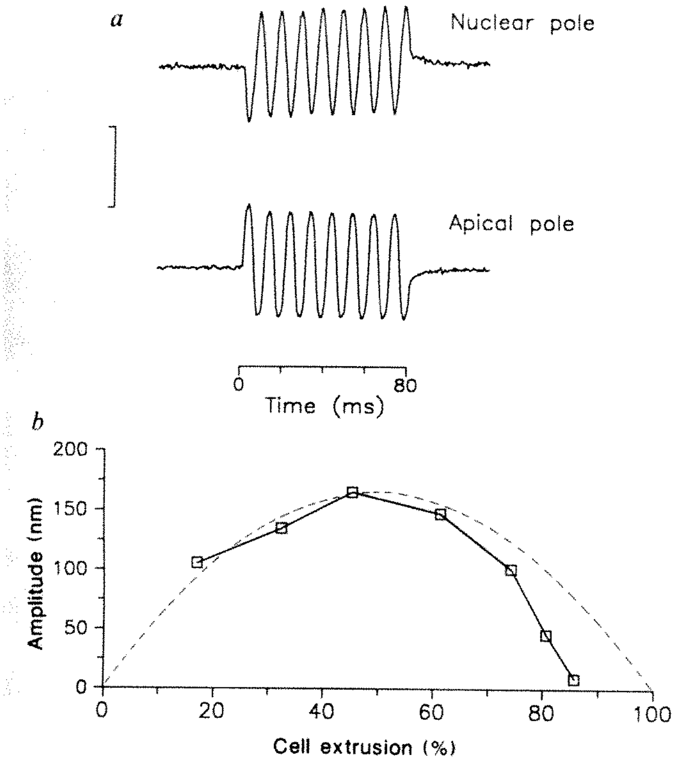


FIG. 3 *a*, Displacement of cell's included basal pole (top) and its extruded apical pole (bottom) in response to 8 cycles of 100-Hz sinusoidal stimulating voltage applied between inside of microchamber and the bath ($V = 125$ mV, peak). The cell is inserted 50%; consequently, the approximate voltage drop on either segment is one-half the applied voltage. Cell length is 65 μm. Elongation is plotted upward, contraction downward. Scale bar, 400 nm. While the extruded pole contracts, the included one extends and vice versa. Depolarization of either membrane segment results in contraction of the associated half of the cell, hyperpolarization results in extension. *b*, Displacement magnitude of the cell's basal pole as the function of fractional cell extrusion, p, for a constant applied stimulus of $V = 62.5$ mV. Cell length, 75 μm. Maximal amplitude of motion occurs at approximately $p = 0.45$. There is no motion of the basal pole for very large extrusions, coincident with the region of the cell that is devoid of subsurface cisterns (L_2 in Fig. 1*c*). To emphasize the latter point, the ideal pattern, $p(1-p)$, normalized to the maximum value of the data, is indicated in the figure with dotted lines. METHODS. Measurement of cell movement is accomplished by passing the image of the cell's nuclear (or ciliated) pole through a rectangular slit onto a photodiode. Cell movement modulates the light flux falling on the photodiode. All measurements are calibrated by use of an optical lever to translate the cell's image in the slit by a known amount[26]. The image of the slit and that of the entire cell are continuously displayed on a split-screen television and recorded on video tape.

would be uniform for the whole cell, predicting that length changes for each segment would be in phase for any p. In Fig. 3*b* we plot the magnitude of motile response measured for the basal (included) pole of a cell, in response to a standard stimulus, with variable extrusion, p. It is apparent that maximal response occurs at some intermediate value of p. A similar result is obtained for the cell's other pole. We have seen no exception to this behaviour in over 20 cells studied. The critical observation, for the present purpose, is that maximal motility occurs somewhere towards the midpoint, $p \approx 0.5$, where the two cell segments, included and excluded, move in opposite phase. It has been shown that outer hair cells are normally under a positive intracellular resting pressure[5] and, moreover, that this pressure is necessary for normal electromotility[6]. We do not dispute these findings, rather we state that change about this resting pressure is not an intermediate step of electromotility. It is further emphasized that the quantitative dependence of the motile response on the degree of cell extrusion from the microchamber is the strongest indication yet obtained of the distributed nature of the elementary motors. Because antiphasic responses between included and extruded segments can be elicited independent of the point of partitioning anywhere within the cell's supranuclear region, the motors must function independently and the length change of a cell or cell segment is simply the sum of the length changes of the individual motor elements. The findings rule out mechanisms of motility that depend on longitudinal voltage gradients such as electro-osmosis[21] and certain forms of electrophoresis[22].

The experiments described here clearly demonstrate that outer hair cell electromotility is driven by a large number of small, independent, voltage-sensing[7] elementary motors, closely associated with the plasma membrane, whose primary mode of action is longitudinal deformation, as shown schematically in Fig. 2*c*. The anatomical basis for the mechanism remains unknown. It is conceivable that transmembrane voltage gradients produce conformational changes in protein structures associated with the cell's cortex that directly change the longitudinal length of the elementary motor units. Longitudinally arranged crossbridges[24] linking helical circumferential filaments ('springs'[13]) of the cortical lattice[14] are ideally situated to convey or generate forces in the axial direction, as has been suggested[24]. This possibility is consistent with the distributed nature of the motor elements and the summation of their contributions[6,11,25] and their association with the cell cortex in contrast to some intracellular structure[6,25]. Because small changes in diameter are also seen during electromotility, it is conceivable that the motors and their attachments are so arranged that they yield vectorial force components in both axial and radial directions. That the elementary motors primarily act in the longitudinal direction implies that they are in effect connected 'in series'. In the condition of these experiments, in the absence of an external mechanical load, the cell behaves as a displacement generator and it is conceivable that it may function in a similar manner *in vivo*. □

Received 29 October; accepted 11 December 1990.

1. Davis, H. *Hearing Res.* **9,** 79–90 (1983).
2. Dallos, P. in *Contemporary Sensory Neurobiology* (eds Correia M. J. & Perachio A. A.) 207–230 (Alan R. Liss, New York, 1985).
3. Kim, D. O. *Hearing Res.* **22,** 105–114 (1986).
4. Brownell, W. E. in *Mechanisms of Hearing* (eds Webster W. R. & Aitkin L. M.) 5–10 (Monash Univ. Press, Clayton, Australia, 1983).
5. Brownell W. E., Bader, C. R., Bertrand, D. & de Ribaupierre, Y. *Science* **227,** 194–196 (1985).
6. Holley, M. C. & Ashmore, J. F. *Proc. R. Soc.* B**232,** 413–429 (1988).
7. Santos-Sacchi, J. & Dilger, J. P. *Hearing Res.* **35,** 143–150 (1988).
8. Ashmore, J. F. *J. Physiol., Lond.* **388,** 323–347 (1987).
9. Lackie, J. M. *Cell Movement and Cell Behaviour* (Allen & Unwin, London, 1986).
10. Saito, K. *Cell Tiss. Res.* **229,** 467–481 (1983).
11. Evans, B. N. *Hearing Res.* **45,** 265–282 (1990).
12. Flock, Å., Flock, B. & Ulfendahl, M. *Arch. Otorhinolaryngol.* **243,** 83–90 (1986).
13. Holley, M. C. & Ashmore, J. F. *Nature* **335,** 635–637 (1988).
14. Bannister, L. H., Dodson, H. C., Astbury, A. F. & Douek, E. E. *Prog. Brain. Res.* **74,** 213–219 (1988).
15. Smith, C. A. & Dempsey, E. W. *Am. J. Anat.* **100,** 337–367 (1957).
16. Guinan, J. J., Warr, W. B. & Norris, B. E. *J. comp. Neurol.* **221,** 358–370 (1983).
17. Spoendlin, H. *Acta Otolaryngol. (Stockholm)* **67,** 239–254 (1969).
18. Dallos, P. & Harris, D. M. *J. Neurophysiol.* **41,** 365–383 (1978).
19. Dallos, P. in *Auditory Function* (eds Edelman G. M., Gall W. E. & Cowan W. M. 153–188 (J. Wiley, New York, 1988).
20. Brownell, W. E. & Kachar, B. in *Peripheral Auditory Mechanisms* (eds Allen J. B., Hall J. L., Hubbard A., Neely S. T. & Tubis A.) 369–376 (Springer-Verlag, New York, 1986).
21. Brownell, W. E. *Ear and Hearing* **11,** 82–92 (1990).
22. Jen, D. H. & Steele, C. R. *J. acoust. Soc. Amer.* **82,** 1667–1678 (1987).
23. Baylor, D. A., Lamb, T. D. & Yau, K.-W. *J. Physiol., Lond.* **288,** 589–611 (1979).
24. Holley, M. C. & Ashmore, J. F. *J. Cell Sci* **96,** 283–291 (1990).
25. Evans, B. N. *thesis,* Univ. Texas Health Sciences Center, Houston (1988).
26. Clark, B. A., Hallworth, R. & Evans, B. N. *Pflügers Arch.* **415,** 490–493 (1990).

ACKNOWLEDGEMENTS. We thank M. A. Cheatham, B. Clark, S. Echteler, M. Killion and J. Siegel for their comments on the manuscript. This work is supported by the NIH and the American Hearing Research Foundation.

Tyrosine phosphorylation and tyrosine kinase activity of the *trk* proto-oncogene product induced by NGF

David R. Kaplan*, Dionisio Martin-Zanca†‡ & Luis F. Parada†

* Eukaryotic Signal Transduction Group
† Molecular Embryology Group, ABL-Basic Research Program, NCI-Frederick Cancer Research and Development Center, PO Box B, Frederick, Maryland 21701, USA
‡ Present address: Departamento de Microbiologia, Universidad de Salamanca, 37008 Salamanca, Spain

NERVE growth factor (NGF) is a neurotrophic factor responsible for the differentiation and survival of sympathetic and sensory neurons as well as selective populations of cholinergic neurons[1,2]. NGF binds to specific cell-surface receptors but the mechanism for transduction of the neurotrophic signal is unknown. Several experiments using the NGF-responsive pheochromocytoma cell line, PC12, have implicated tyrosine phosphorylation in NGF-mediated responses, although no NGF-specific tyrosine kinases have been identified[3]. Here we show that NGF induces tyrosine phosphorylation and tyrosine kinase activity of the *trk* proto-oncogene product, a tyrosine kinase receptor whose expression is restricted *in vivo* to neurons of the sensory spinal and cranial ganglia of neural crest origin[4]. Tyrosine phosphorylation of *trk* by NGF is rapid, specific and occurs with picomolar quantities of factor, indicating that the response is mediated by physiological amounts of NGF. Activation of the *trk* tyrosine kinase receptor provides a possible mechanism for signal transduction by NGF.

In the presence of NGF, PC12 cells differentiate into sympathetic neurons, extend neurites, increase neurotransmitter synthesis and become electrically excitable[5-7]. Several unidentified proteins are phosphorylated on tyrosine in these cells and the introduction of the tyrosine kinase v-*src* into PC12 cells stimulates an outgrowth of neurites similar to that resulting from NGF treatment[3,8]. Therefore, events mediated by tyrosine phosphorylation could be involved in the differentiation of PC12 cells.

The *trk* gene is a member of a gene family of tyrosine kinase (TK) receptors that includes the related gene *trk*b[4,9,10]. To determine if *trk* or *trk*b were transcribed in PC12 cells, the expression of *trk* transcripts was assayed by northern-blot transfer analysis with a full-length *trk* complementary DNA probe or with an extracellular domain, *trk*b-specific probe[9]. PC12 cells expressed a 3.2-kilobase *trk* transcript, but *trk*b transcripts were not detected (Fig. 1*a*). The level of *trk* transcripts was not affected by the addition of NGF. To determine whether additional *trk*-related genes were transcribed in PC12 cells, messenger RNA was hybridized at low stringency with the highly conserved *trk* TK domain. No additional *trk*-hybridizing transcripts were detected (not shown).

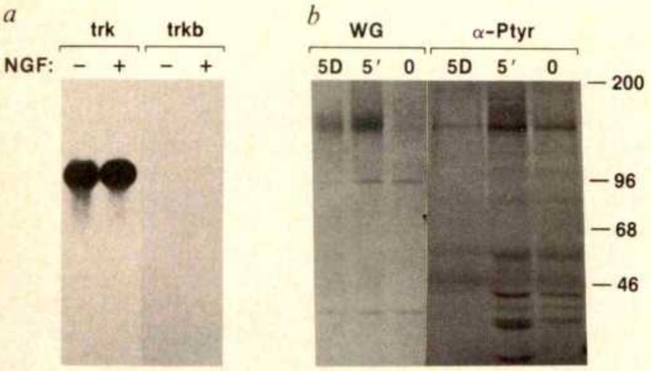


FIG. 1 *a*, Northern-blot transfer analysis of *trk* and *trk*b transcripts in NGF treated (+) or untreated (−) PC12 cells. *b*, Ptyr: phosphotyrosine-containing proteins in PC12 cells treated for 5 min or 5 days with NGF or in untreated cells (0). WG, tyrosine-phosphorylated glycoproteins in cells treated with NGF for 5 min or 5 days. M_r markers (in K) are indicated.

METHODS. *a*, RNA preparation and northern blot transfer analysis was performed as described previously[4]. Cells were treated with 50 ng per ml NGF(+) (Boehringer-Mannheim) and were collected 48 h later after differentiation had occurred. Total RNA (20 μg) was loaded per lane and the filter was hybridized with a probe specific for *trk*[4] or *trk*b[9]. *b*, Ptyr; PC12 cells were treated with 50 ng per ml NGF for 5 min or 5 days. Cells were washed, lysed and the lysates (50 μg protein per lane) analysed by immunoblotting with the phosphotyrosine (Ptyr) monoclonal antibody 4G10 (gift from D. Morrison, B. Drucker and T. M. Roberts) as previously described[17,18]. For WG, lysates (prepared from 2×10^7 cells) were incubated with 100 μl of a 50% solution of wheat-germ (WG) lectin agarose (Pharmacia) for 2 h at 4 °C. Precipitates were washed and analysed by western blot analysis with anti-Ptyr as previously described[17,18]. Electrophoresis was performed on 7.5% SDS-polyacrylamide gels.

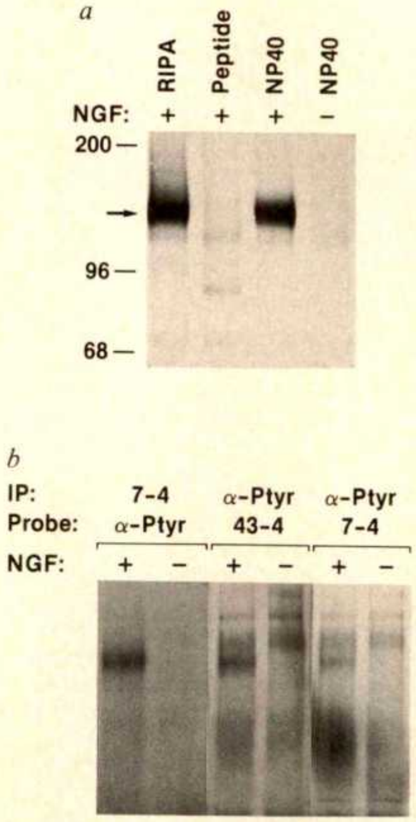


FIG. 2 NGF induces tyrosine phosphorylation of Trk. *a*, Trk was immunoprecipitated with rabbit peptide antibody 43-4[11] from lysates of NGF-treated (+) or untreated (−) PC12 cells and the immunoprecipitates were probed with anti-Ptyr. Cells were lysed in buffer containing 1% NP40[18] or RIPA buffer[19]. Immunoprecipitations were performed in the presence or absence of competing peptide (10 μg ml^{-1}). Similar results were obtained with other sources of NGF (Collaborative Research, Upstate Biotechnology Inc.). *b*, NGF-induced tyrosine phosphorylation of Trk in anti-Trk or anti-Ptyr immunoprecipitates. Trk immunoprecipitates were prepared with rabbit antibody 7-4[11] before anti-Ptyr western blot analysis or with Ptyr antibody before western blot analysis with Trk antibodies 43-4 or 7-4.

METHODS. PC12 cells were treated for 5 min at 37 °C with 50 ng per ml NGF. Cells (2×10^7) were lysed in buffer containing 1% Nonidet P-40 (NP40) (except as noted) and were immunoprecipitated with anti-Trk or anti-Ptyr for 2–3 h at 4 °C as described previously[18]. Immunoprecipitates were collected with protein-A/Sepharose, subjected to 7.5% SDS-PAGE and analysed by immunoblotting as described[18]. M_r markers (in K) are indicated.

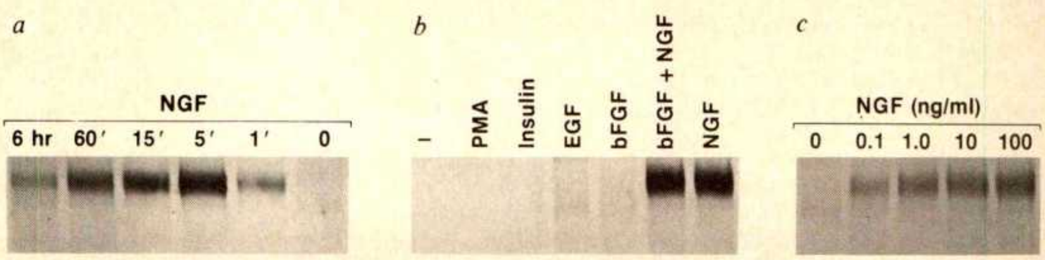


FIG. 3 Time course, growth factor specificity and dose-response of Trk tyrosine phosphorylation in PC12 cells. *a*, Time course of Trk tyrosine phosphorylation. Cells (2×10^7) were treated with 50 ng per ml NGF at 37 °C. *b*, Effects of growth and differentiation factors on Trk tyrosine phosphorylation. Cells were treated with 100 ng per ml NGF, 100 ng per ml basic fibroblast growth factor (bFGF) (Boehringer-Mannheim), 100 ng per ml epidermal growth factor (EGF) (Upstate Biotechnology, Inc.), 100 nM insulin (Sigma), or 1 μg per ml phorbol 12-myristate 13-acetate (PMA) (Sigma) for 5 min at 37 °C. *c*, Dose-response of Trk tyrosine phosphorylation. Cells were treated for 30 min at 37 °C with increasing concentrations of NGF. Western blot analysis with Ptyr antibodies of Trk immunoprecipitates prepared with anti-Trk antibody 43-4 are shown.

Phosphotyrosine (Ptyr) western-blot transfers of cell lysates prepared from PC12 cells treated with NGF for 5 min demonstrated the tyrosine phosphorylation of a broad band at relative molecular mass 130,000–140,000 (M_r 130–140K) (Fig. 1*b*). Proteins of 42, 45, 50, 60 and 150K were also observed to be phosphorylated on tyrosine in response to NGF treatment of PC12 cells. A band of 130–140K was observed when the cell lysates were precipitated with wheat-germ lectin before analysis by anti-Ptyr immunoblotting, indicating that NGF induced the tyrosine phosphorylation of a glycosylated protein (Fig. 1*b*).

To determine whether Trk, a 140K glycoprotein[11], was phosphorylated on tyrosine in response to NGF, Trk protein was immunoprecipitated with an antibody generated against a C-terminal Trk peptide (43-4)[11] and the immunoprecipitates were probed with anti-Ptyr antibodies. Trk was phosphorylated on tyrosine after addition of NGF to the cells (Fig. 2*a*). Immunoprecipitation of Trk was specifically blocked by a Trk-derived peptide and was not reduced by immunoprecipitation in stringent buffers such as RIPA (Fig. 2*a*). Tyrosine phosphorylation of Trk was also observed when the immunoprecipitations were performed using a second anti-Trk antibody generated against the $p70^{trk}$ oncogene product (7-4)[11] and when anti-Ptyr immunoprecipitates prepared from NGF-treated cells were probed with either of two anti-Trk antibodies (Fig. 2*b*). The same amount of Trk protein was recovered in each immunoprecipitation, as measured by [^{35}S] methionine labelling or Trk (not shown). To confirm that Trk was phosphorylated on tyrosine residues in response to NGF, PC12 cells were labelled with ^{32}P-orthophosphate before NGF addition and immunoprecipitation with anti-Trk antibodies. Phosphoamino-acid analysis demonstrated that Trk was phosphorylated on tyrosine in response to NGF (not shown).

Trk tyrosine phosphorylation occurred within 1 min of NGF treatment of cells, reached maximum levels after 5 min and declined thereafter (Fig. 3*a*). Residual phosphorylation was detected after two days of treatment with NGF when the cell population was fully differentiated (not shown). Trk tyrosine phosphorylation was also specific to NGF. Other peptide growth factors that elicit tyrosine phosphorylation in PC12 cells were tested in our assay[3,12]. Epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), insulin and the phorbol ester phorbol 12-myristate 13-acetate (PMA) failed to induce Trk tyrosine phosphorylation (Fig. 3*b*). No additional tyrosine phosphorylation of Trk was seen in cells treated with both bFGF and NGF (Fig. 3*b*). These agents produce similar patterns of early responses in PC12 cells, including transcriptional activation of c-*fos* and c-*myc*[13]. But of these factors, only NGF and bFGF stimulate neurite outgrowth.

To determine the minimal concentration of NGF required for Trk tyrosine phosphorylation, a dose-response experiment was performed. Tyrosine phosphorylation was half-maximal at 0.1 ng per ml NGF (50 pM) (Fig. 3*c*), indicating that Trk phosphorylation occurs at physiologically relevant concentrations of NGF[14].

NGF also induces the tyrosine kinase activity of Trk. Trk was phosphorylated *in vitro* in immunoprecipitates prepared from PC12 cells treated for 5 min with NGF, presumably owing to catalytic autophosphorylation (Fig. 4). No Trk phosphorylation was detected in immunoprecipitates from untreated cells or when competing Trk-derived peptide was included during immunoprecipitation.

Our results show that the protein tyrosine kinase receptor Trk is a substrate for an NGF-induced tyrosine kinase activity in PC12 cells. The rapid and specific response suggests that Trk is closely associated with the receptor for NGF. Alternatively, the Trk protein may directly bind NGF. The kinase activity of Trk

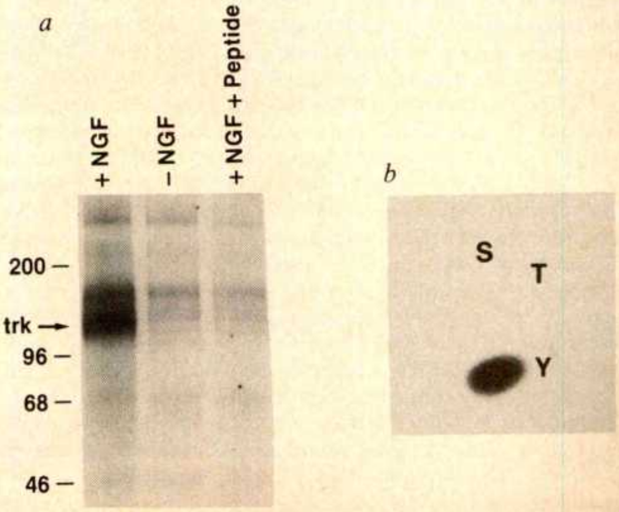


FIG. 4 Activation of Trk kinase activity by NGF. *a*, Immune-complex kinase assay following immunoprecipitation with anti-Trk antibodies prepared from NGF-treated (+) or untreated (–) PC12 cells. Immunoprecipitations were performed in the presence or absence of competing peptide (10 μg ml^{-1}). *b*, Phosphoamino-acid analysis autoradiogram of Trk phosphorylated *in vitro* in *a*. The positions of serine (S), threonine (T) and tyrosine (Y) are indicated. METHODS. PC12 cells (8×10^7) were lysed and precipitated with wheat-germ lectin-agarose (300 μl 50% solution) as for Fig. 1. The precipitates were washed three times in 1% NP40-containing lysis buffer[18] and the glycoproteins were eluted in 400 μl 0.5 M *N*-acetylglucosamine in lysis buffer containing 1 mM orthovanadate for 1 h at 4 °C. The eluate was diluted 10-fold with lysis buffer and immunoprecipitated with anti-Trk antibody as described in Fig. 2 legend. The immunoprecipitates were incubated with 20 μCi [γ-^{32}P] ATP, 10 mM $MnCl_2$, 20 mM Tris, pH 7.4, for 20 min at 25 °C. Phosphorylated proteins were analysed by 7.5% SDS-PAGE as described[18]. M_r markers (in K) are indicated. For *b*, the phosphorylated Trk band was eluted from the gel and phosphoamino-acid analysis performed as previously described[21].

may also be activated by phosphorylation of Trk by another cellular tyrosine kinase.

Trk may be responsible for the increases in phosphotyrosine in NGF-treated PC12 cells. Several proteins phosphorylated on tyrosine in these cells are candidate substrates for the Trk-induced kinase activity, including microtubule associated protein-2 (MAP-2) kinase and phospholipase C_γ[15] (M. Vetter, D.M.-Z., L.F.P., J. M. Bishop and D.R.K., manuscript submitted). Examination of Trk immunoprecipitates for co-immunoprecipitating substrates should facilitate the identification of other targets of tyrosine kinase activities in PC12 cells.

The expression *in vivo* of *trk* is consistent with its proposed role in NGF-mediated events. The *trk* mRNA is restricted to the dorsal root ganglia and cranial sensory ganglia of neural crest origin, tissues comprised primarily of neurons that are trophic substrates for NGF[4]. These neurons rely on NGF for differentiation and maintenance of the neuronal phenotype[16]. Sympathetic neurons and certain neurons in the central nervous system, which may not express *trk*, also respond to NGF[1,2]. But other *trk* family members, for example *trk*b, are expressed in the central nervous system[9,10] and may function as NGF-responsive tyrosine kinases in these tissues. Other tyrosine kinases expressed in neurons, including $p60^{c\text{-}src}$ and $p62^{c\text{-}yes}$, may be involved in NGF responses, although the phosphorylation state and kinase activity of these proteins does not seem to be altered in response to addition of NGF (M. Vetter, D.K. and M. J. Bishop, unpublished results). Trk is a likely candidate for a transducer of NGF signals. These results implicate tyrosine kinase receptors as possible mediators of neurotrophic signals in vertebrates. ☐

Received 18 January; accepted 12 February 1991.

1. Levi-Montalcini, R. *Science* **237,** 1154–1161 (1987).
2. Gnahn, H., Heft, F., Heumann, R., Schwab, M. E. & Thoenen, H. *Devl Brain Res.* **9,** 45–52 (1983).
3. Maher, P. A. *Proc. natn. Acad. Sci. U.S.A.* **85,** 6788–6791 (1988).
4. Martin-Zanca, D., Barbacid, M. & Parada, L. F. *Genes Dev.* **4,** 683–694 (1990).
5. Greene, L. A. & Tischler, A. S. *Proc. natn. Acad. Sci. U.S.A.* **73,** 2424–2428 (1976).
6. Schubert, D., Heinemann, S. & Kidokoro, Y. *Proc. natn. Acad. Sci. U.S.A.* **74,** 2579–2583 (1977).
7. Dichter, M. A., Tischler, A. S. & Greene, L. A. *Nature* **268,** 501–504 (1977).
8. Alema, S., Casalbore, P., Agostini, E. & Tato, F. *Nature* **316,** 557–559 (1985).
9. Klein, R., Martin-Zanca, D., Barbacid, M. & Parada, L. F. *Development* **4,** 845–850 (1990).
10. Klein, R., Parada, L. F., Coulier, F. & Barbacid, M. *EMBO J.* **8,** 3701–3709 (1989).
11. Martin-Zanca, D., Oskam, R., Mitra, G., Copeland, T. & Barbacid, M. *Molec. cell. Biol.* **9,** 24–33 (1988).
12. Maher, P. A. *J. Neurosci. Res.* **24,** 29–37 (1989).
13. Greenberg, M. E., Hermanowski, A. L. & Ziff, E. B. *Molec. cell. Biol.* **6,** 1050–1057 (1986).
14. Greene, L. A. & Rein, G. *Nature* **268,** 349–351 (1977).
15. Kim, U.-H. *et al. J. biol. Chem.* (in the press).
16. Thoenen, H. & Barde, Y. A. *Physiol. Rev.* **60,** 1284–1335 (1980).
17. Morrison, D. K., Browning, P. J., White, M. F. & Roberts, T. M. *Molec. cell. Biol.* **8,** 176–185 (1988).
18. Kaplan, D. R., Morrison, D. K., Wong, G., McCormick, F. & Williams, L. T. *Cell* **61,** 125–133 (1990).
19. Morrison, D. K. *et al. Cell* **58,** 649–657 (1989).
20. Chao, M. V. in *Handbook of Experimental Pharmacology* Vol. 95/II (eds Sporn, M. B. & Roberts, A. B.) 135–165 (Springer-Verlag, 1990).
21. Sefton, B. M., Hunter, T., Ball, E. H. & Singer, S. J. *Cell* **24,** 165–174 (1981).

ACKNOWLEDGEMENTS. We thank D. Morrison for phosphoamino acid analysis, H. Varmus for support and advice, and M. Chao, B. Hempstead and M. Vetter for helpful discussions. We also thank N. Copeland, S. Hughes, M. Chao and B. Hempstead for critically reading the manuscript, J. Maragos and S. Rabin for technical help, and R. Handley for assistance in preparing the manuscript. This research was supported by the National Cancer Institute, DHHS, under contract.

A cellular protein that competes with SV40 T antigen for binding to the retinoblastoma gene product

Shi Huang, Wen-Hwa Lee & Eva Y.-H. P. Lee*

Department of Pathology, School of Medicine, University of California,
San Diego, La Jolla, California 92093-0612, USA

TUMOUR-suppressor genes, such as the human retinoblastoma susceptibility gene (*Rb*), are widely recognized as being vital in the control of cell growth and tumour formation[1]. This role is indicated, in part, by the suppression of tumorigenicity of human tumour cells after retrovirus-mediated *Rb* replacement[2–4]. How *Rb* acts to bring about this suppression is not clear[5] but one clue is that the Rb protein forms complexes with the transforming oncoproteins of several DNA tumour viruses[6–8], and that two regions of Rb essential for such binding frequently contain mutations in tumour cells[9,10]. These observations suggest that endogenous cellular proteins might exist that bind to the same regions of Rb and thereby mediate its function. We report here the identification of one such human cellular Rb-associated protein of relative molecular mass 46,000 (46K) (RbAP46). Two lines of evidence support the notion that RbAP46 and simian virus 40 T antigen have homologous Rb-binding properties: first, several mutated Rb proteins that failed to bind to T also did not associate with RbAP46; and second, both T antigen and T peptide (amino acids 101–118) were able to compete with RbAP46 for binding to Rb. The apparent targeting of the RbAP46–Rb interaction by oncoproteins of DNA tumour viruses strongly suggests that formation of this complex is functionally important.

Previous attempts to identify Rb-associated proteins[11] may have been hampered by insufficient quantities of such stable complexes in cells. One approach to overcome this difficulty is to add excess free Rb protein *in vitro*, thereby driving complex formation. Detectable amounts of RbAPs could then be coprecipitated by anti-Rb antibody. To obtain large quantities of Rb protein, we constructed plasmid pETRbc, containing the entire coding region of Rb, for high-level expression in *Escherichia coli*[12] (Fig. 1*a*). The most stably expressed protein was a 56K polypeptide (p56-Rb), which was then purified (Fig. 1*b*) and characterized immunologically (Fig. 1*c*). Only antibodies recognizing the C-terminal half of Rb could precipitate this protein, whereas two different antibodies recognizing the N-terminal regions failed to precipitate it. The C-terminal 56K contains both regions essential for T binding[9,10]. When purified T antigen was added to bacterial lysates, this 56K polypeptide was coprecipitated by anti-T antibody (data not shown). Conversely, mixing increasing amounts of purified 56K protein with radiolabelled COS cell lysates, in which T is expressed in great excess over endogenous Rb[7], resulted in quantitative coprecipitation of T by anti-Rb antibody over a 10-fold range (data not shown). Similar results were obtained by mixing COS cell extracts with lysates of bacteria expressing Rb protein (Fig. 3, lanes 1 and 2). These studies indicated that bacterially expressed p56-Rb retained the T-binding properties of full-length Rb.

We next tested whether a cellular protein exists in human HeLa cell extracts that binds to p56-Rb in a similar way. As shown in Fig. 2*a*, a protein of 46K (RbAP46) was specifically detected only when p56-Rb, either in bacterial lysates (Fig. 2*a*, lane 2) or in a purified form (Fig. 2*a*, lanes 4–7), was mixed with radiolabelled HeLa cell lysates. By mixing increasing amounts of purified p56-Rb with constant amounts of HeLa cell lysates, the 46K protein was immunoprecipitated in increasing amounts (Fig. 2*a*, lanes 3–7), suggesting that it formed a complex with the input 56K protein. To exclude the possibility of cross-reaction of the 46K protein with the rabbit anti-Rb 0.495, another anti-Rb antibody, 0.47, was used[10], and again the 46K protein was coprecipitated (Fig. 2*b*, lane 11). But no 46K protein was detected if nonimmune serum was used (Fig. 2*b*, lane 9). These results implied that this 46K protein was directly associated with p56-Rb. Using a full-length Rb protein expressed in insect cells[13], the same 46K protein was again coprecipitated (Fig. 2*c*, lane 15), although some additional protein bands were also seen. As RbAP46 was the major protein detected by using either a full-length Rb or p56-Rb, we concluded that p56-Rb contains all the necessary sequences for binding to the 46K protein.

The specificity of the interaction was further characterized by defining the regions of Rb involved in binding to RbAP46. Five

---

* To whom correspondence should be addressed.

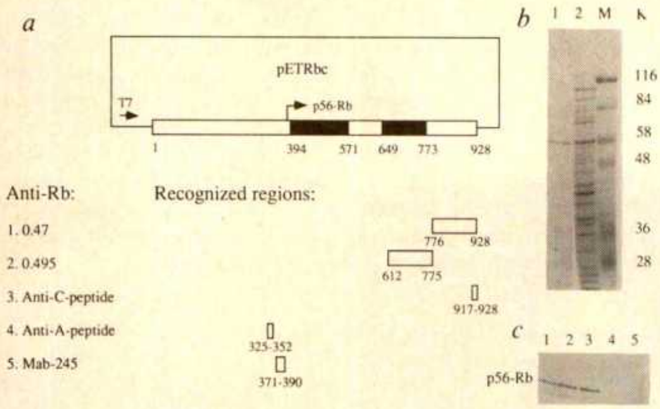


FIG. 1 Expression of Rb polypeptides in *E. coli*. *a*, Maps of plasmid pETRbc and regions of Rb protein recognized by various Rb antibodies. T-binding regions of Rb are represented as filled squares. *b*, Purification of p56-Rb. Bacterial lysate containing p56-Rb (lane 2) and one of the peak fractions collected from CM-Sepharose chromatography (lane 1) were analysed by 10% SDS-PAGE and stained with Coomassie blue. Lane M shows migration of markers, molecular weights indicated (in thousands) to the right. *c*, Immunological characterization of p56-Rb. Partially purified p56-Rb was immunoprecipitated with various anti-Rb antibodies (lane 1, 0.47; lane 2, 0.495; lane 3, anti-Rb c-peptide (amino acids 917-928); lane 4, anti-Rb A-peptide (amino acids 325-352); lane 5, Monoclonal antibody (Mab-245), and the immunoprecipitates were analysed by western blotting with anti-Rb antibody 0.47[10,17,18].

METHODS. The *Nco*I–*Bam*HI fragment of the full-length Rb coding region derived from pB10[10] was inserted into *Nco*I–*Bam*HI sites of pET8c vector[12]. The resulting plasmid pETRbc was transformed into host BL21(DE3)pLysS. Transformed *E. coli* cells were grown in LB media containing 100 μg ml^{-1} ampicillin and 25 μg ml^{-1} chloramphenicol, and induced by 0.4 mM isopropylthiogalactoside (IPTG) when the culture reached an optical density at 600 nm of 0.6–1. After 2-h induction, 1 l of culture was collected and lysed in lysis buffer (50 mM Tris–HCl, pH 7.5, 100 mM NaCl, 2 mM EDTA containing 0.5 mM PMSF) by freezing and thawing. The lysate was clarified by centrifugation at 35,000 r.p.m. for 3 h and the supernatant was fractionated on a 1×15-cm CM-Sepharose CL6B column (Pharmacia) by salt gradient elution (0.1–0.5 M KCl). The fractions enriched to about 80% purity were collected and used for subsequent experiments. Immunological characterization of partially purified p56-Rb was done as previously described[18].

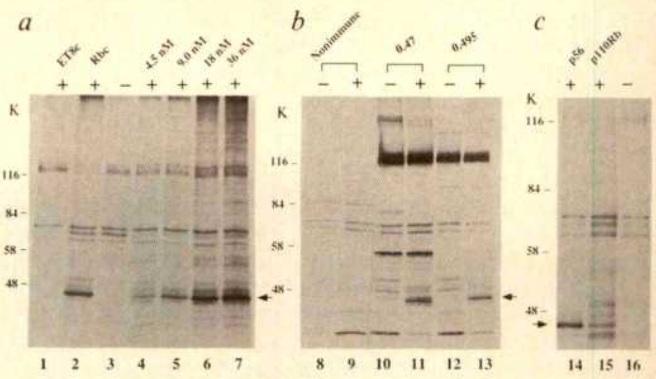


FIG. 2 Detection of RbAP46 in HeLa cell extracts. *a*, Radiolabelled HeLa cell lysates were mixed with the following samples before immunoprecipitation with anti-Rb 0.495: lane 1, control bacterial lysate from IPTG-induced pET8c transformants; lane 2, lysate from IPTG-induced pETRbc transformants; lane 3, buffer alone; lanes 4–7, increasing amounts of purified p56–Rb as indicated. *b*, HeLa cell lysates were mixed with 0.5 μg purified p56–Rb (lanes 9, 11 and 13) or buffer alone (lanes 8, 10 and 12), and immunoprecipitated with nonimmune serum (lanes 8 and 9), anti-Rb 0.47 (lanes 10 and 11), and anti-Rb 0.495 (lanes 12 and 13). *c*, HeLa cell lysates were mixed with 0.5 μg either purified p56–Rb (lane 14) or purified p110–Rb (lane 15) or buffer alone (lane 16) and immunoprecipitated by antibody 0.495. Immunoprecipitates were analysed by 10% SDS–PAGE. Molecular weight calibrations (in K) are shown to the left. Arrows point to the 46K protein.

METHODS. Cells grown nearly confluent were labelled with [^{35}S] methionine for 3 h, washed with cold PBS and then lysed in 1 ml EBC buffer (50 mM Tris-HCl, pH8.0, 120 mM NaCl, 0.5% Nonidet P-40) containing 200 U ml^{-1} aprotinin and leupeptin and 1 mM phenylmethylsulphonyl fluoride. The lysates were clarified by centrifugation and aliquots (0.5 ml) of supernatants were taken. Different Rb proteins or control samples were then mixed with each 0.5 ml lysate for 90 min on ice and then incubated with 2 μg anti-Rb or control antibody for 40 min. Immunocomplexes were collected by adding 80 μl protein A-Sepharose beads in TBS-BSA buffer for 40 min. After washing five times with 1 ml NET-N buffer (20 mM Tris–HCl, pH8.0, 100 mM NaCl, 1 mM EDTA, 0.5% NP-40), the beads were boiled in SDS sample buffer and analysed by SDS–PAGE and fluorography.

deletion or insertion mutants of p56–Rb were constructed as previously described[10] and expressed in *E. coli* using the T7 expression system (Fig. 3*b*). Crude bacterial extracts were prepared, and the levels of p56–Rb derivatives in each lysate were determined by western blotting analysis. To examine the T-binding properties of these Rb mutants, bacterial lysates containing equivalent amounts of Rb were mixed with radiolabelled COS cell lysates and the mixture was immunoprecipitated with anti-Rb antibody. Although T antigen was coprecipitated with endogenous Rb in COS cells (Fig. 3*a*, lane 1), addition of wild-type p56–Rb or mutant pETM9 were able to bring down more T antigen than that associated with endogenous Rb (Fig. 3*a*, compare lanes 2 and 6 with lane 1). Addition of four other mutant proteins actually reduced the amount of coimmunoprecipitating T antigen because the level of anti-Rb antibody was limiting in this analysis. These results essentially replicated our previous definition of the T-binding domains of Rb protein[9,10]. Interestingly, when the same set of the mutant Rb proteins were added to radiolabelled HeLa cell lysates, the 46K protein coprecipitated with wild-type p56–Rb and pETM9,

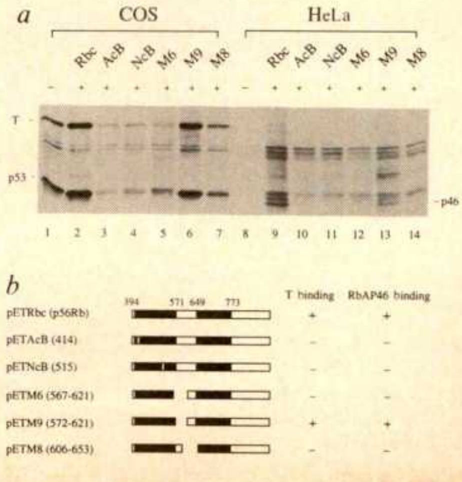


FIG. 3 Defining regions of Rb required for association with T and RbAP46. *a*, Radiolabelled COS or HeLa cell lysates were mixed with bacterial lysates containing equivalent amounts of p56–Rb derivatives and immunoprecipitated with anti-Rb 0.47. In COS cells, p53 appeared because it is known to be associated with T, and RbAP46 is not seen because its binding is blocked by excess T. *b*, Structure of the various mutant p56–Rb polypeptides. A schematic representation of p56–Rb is shown on top of the mutant polypeptides. The N-terminal boundary of p56–Rb is not precisely determined yet.

METHODS. Plasmids pETAcB and pETNcB were constructed by inserting the small *Mlu*I–*Hpa*I fragments derived from digestion of pAcB and pNcB[10] respectively into the corresponding region of pETRbc. Similarly, pETM6, pETM8 and pETM9 were constructed by inserting the small fragments derived from *Hpa*I and *Dra*III digestions of plasmids pM6, pM8 and pM9[10] into pETRbc at its corresponding sites. Transformants were grown in 10-ml culture and collected 2 h after induction with isopropylthiogalactoside. Cells were lysed in 1 ml lysis buffer by freezing and thawing, and lysates were spun in a microfuge to clear cell debris. The supernatant was used directly for the mixing experiments.

but not or very weakly with the other four mutants (Fig. 3*a*, lanes 8–14). These results indicated that the regions of Rb involved in RbAP46 binding coincide precisely with the two regions necessary for binding to T.

If the 46K protein indeed binds to the same domain of Rb as does T, then T antigen should compete effectively with RbAP46 for binding to Rb. In addition, T antigen should be replaceable in competition studies by T peptide (a synthetic 18-residue polypeptide containing amino-acid residues 101–118 of T antigen which are necessary and sufficient for Rb-binding) but not by mutant K peptide which contains the same sequence but with a Lys → Glu substitution at position 107 (ref. 14). This experiment was performed by mixing HeLa cell lysates with p56–Rb, alone or together with T antigen or peptides. Effective competition was observed with 40 nM T antigen (Fig. 4*a*, lane 4) or 50 μM T peptide (Fig. 4*b*, lane 10), although identical amounts of unrelated proteins (data not shown) or mutant K peptide (Fig. 4*b*, lane 8) were less potent. These results further supported the conclusion that RbAP46 binds to Rb at its T antigen binding domain.

In short, RbAP46 shares the properties and features of Rb binding with T antigen and E1A, oncoproteins of two DNA tumour viruses. Beyond this, the function of the 46K protein and of its association with Rb protein are obscure. It has been proposed that oncoproteins of DNA tumour viruses may transform cells by binding to and inactivating Rb protein. Analogously, one can imagine that the 46K protein may serve as a regulator of Rb to neutralize its biological function under certain cellular conditions. Alternatively, the 46K protein may be a downstream mediator of Rb's normal physiological function. In either case, the lack of RbAP46–Rb complex formation might result in loss of growth control. Consistent with this notion, the regions of Rb involved in T- or RbAP46-binding are common sites for naturally occurring mutations in human tumour cells[10]. For example, we have found that a single amino-acid substitution in exon 21, as in small-cell lung tumour cell line H209[15], abolished binding to both T and RbAP46 (data not shown). Although the biological function of RbAP46 has yet to be defined, the inverse correlation of RbAP46 binding to, and biologically significant mutations of, the Rb gene product strongly suggests that the interaction with RbAP46 plays a pivotal role in normal Rb function.

It is not known whether RbAP46 contacts Rb directly, or if intermediate proteins are involved. RbAP46 seems to be a major protein bound to Rb detected by our method, although other protein species have been inconsistently seen. Perhaps different members of Rb protein can interact with different sets of cellular proteins, depending on cellular growth status. Recently, transcription of the c-*fos* gene has been shown to be downregulated when cells were transfected with Rb[16]. It is plausible that Rb-associated protein complexes could bind directly to the c-*fos* promoter, and RbAP46 is a candidate for membership in such complexes. Further characterization of RbAP46 will certainly replace these provocative speculations with real insight into the molecular mechanisms of tumour suppression. ☐

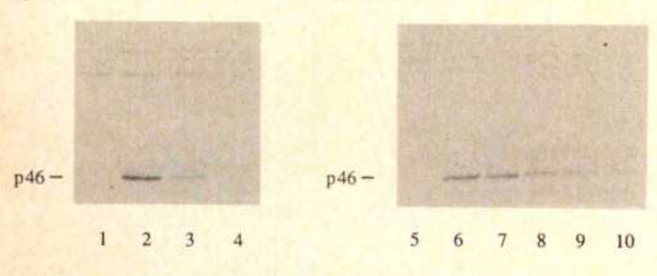

a
T antigen – – 20 nM 40 nM
p56Rb – + + +
p46 –
1 2 3 4
b
T peptide – – – – 5 μM 50 μM
K peptide – – 5 μM 50 μM – –
p56Rb – + + + + +
p46 –
5 6 7 8 9 10

FIG. 4 T antigen and T peptide compete with RbAP46 for binding to Rb. Radiolabelled HeLa cell lysates were mixed with different samples as indicated on top of each lane and incubated for 90 min on ice. Immunoprecipitation was done by anti-Rb 0.495 and subsequently analysed as described in Fig. 2. The amino-acid sequence (one-letter code) of T peptide is ENLFCSEEMPSSDDEAAT, and the K peptide is ENLFCSKEMPSSDDEAAT. These two peptides differ only in one amino acid at position 7. About 20 nM of p56–Rb was used where indicated.

Received 20 September; accepted 21 December 1990.

1. Klein, G. *Science* **238**, 1539–1545 (1987).
2. Huang, H.-J. S. *et al. Science* **242**, 1563–1566 (1988).
3. Bookstein, R., Shew, J.-Y., Chen, P.-L., Scully, P. & Lee, W.-H. *Science* **247**, 712–715 (1990).
4. Sumegi, J., Uzvolgyi, E. & Klein, G. *Cell Growth Differ.* **1**, 247–250 (1990).
5. Lee, W.-H., Bookstein, R. & Lee, E. Y.-H. P. in *Tumor Suppressor Genes* (ed. Klein, G.) 169–200 (Marcel Dekker, New York, 1990).
6. Whyte, P. *et al. Nature* **334**, 124–129 (1988).
7. DeCaprio, J. A. *et al. Cell* **54**, 275–283 (1988).
8. Dyson, N., Howley, P. M., Munger, K. & Harlow, E. *Science* **243**, 934–937 (1989).
9. Hu, Q., Dyson, M. N. & Harlow, E. *EMBO J.* **9**, 1147–1155 (1990).
10. Huang, S., Wang, N.-P., Tseng, B. Y., Lee, W.-H. & Lee, Y.-H. P. *EMBO J.* **9**, 1815–1822 (1990).
11. Xu, H.-J., Hu, S.-X., Hashimoto, T., Takashi, R. & Benedict, W. F. *Oncogene* **4**, 807–812 (1989).
12. Rosenberg, A. H. *et al. Gene* **56**, 125–135 (1987).
13. Wang, N.-P., Qian, Y.-W., Chung, A. E., Lee, W.-H. & Lee, E. Y.-H. P. *Cell Growth Differ.* **1**, 429–437 (1990).
14. DeCaprio, J. A. *et al. Cell* **58**, 1085–1095 (1989).
15. Bignon, Y.-J. *et al. Cell Growth Differ.* **1**, 647–651 (1990).
16. Robbins, P. D., Horowitz, J. M. & Mulligan, R. C. *Nature* **346**, 668–671 (1990).
17. Wang, N.-P. *et al. Cell Growth Differ.* **1**, 233–239 (1990).
18. Shew, J.-Y., Ling, N., Yang, X., Fodstad, O. & Lee, W.-H. *Oncogene Res.* **1**, 205–214 (1989).

ACKNOWLEDGEMENTS. We thank F. W. Studier for plasmids and strains of the T7 expression system, and F. Hong, C. Lin, M. Kapiloff and R. Chen for discussions concerning the use of the system; N.-P. Wang and Y.-W. Qian for p110-Rb from insect cells, and for anti-Rb antibodies 0.495 and 0.47; B. Tseng for SV40 T antigen; N. Lin for peptide synthesis and purification; and R. Bookstein for reading the manuscript. This work was supported by the NIH and Council for Tobacco Research to E. Y.-H.P.L. and W.-H.L. S.H. was supported in part by CRCC, University of California, and by a Fight For Sight—Prevent Blindness Postdoctoral Fellowship.

Alternative temporal control systems for hypodermal cell differentiation in *Caenorhabditis elegans*

Zhongchi Liu & Victor Ambros

Department of Cellular and Developmental Biology, Harvard University,
16 Divinity Avenue, Cambridge, Massachusetts 02138, USA

IN certain multicellular organisms, genetic regulatory systems that specify the timing of cell division, differentiation and morphogenesis[1–3] must accommodate environmental and physiological contingencies that perturb or arrest development. For example, *Caenorhabditis elegans* can either develop continuously through four larval stages (L1–L4) or arrest indefinitely as a 'dauer larva' at the second larval (L2) moult, and later resume L3 and L4 development[4–7]. At the larva-to-adult (L4) moult of both continuous and 'post-dauer' development, hypodermal cells switch (the 'L/A switch') from a proliferating state to the terminally differentiated state. Four temporal regulators, *lin-4*, *lin-14*, *lin-28* and *lin-29*, have been identified in *C. elegans* by mutations that cause precocious or retarded expression of stage-specific post-embryonic development events, including the L/A switch (refs 3, 8, 9; Fig. 1*a*). These genes have been organized into a genetic pathway that controls the timing of the L/A switch during continuous development[10]: *lin-29* activates the switch and the other heterochronic genes regulate it indirectly by regulating *lin-29*. We have now examined how the proper timing of this event is specified in alternative developmental pathways. In continuously developing *lin-4*, *lin-14* and *lin-28* mutants the L/A switch occurs at abnormally early or late moults[3,8], but during post-dauer development of the same mutants the L/A switch occurs normally. Thus

TABLE 1 Expression of the L/A switch in heterochronic mutants during continuous and post-dauer development

Genotype		Heterochronic expression of the L/A switch			
		Precocious		Retarded	
		Continuous	Post-dauer	Continuous	Post-dauer
Wild type		—	—	—	—
lin-14	*n355 n679ts* 15 °c	—	—	100% (21/21)	19%* (4/21)
	n536/+	—	—	100% (*n* > 100)	3%* (2/73)
	n355 n679ts 25 °C	100% (40/40)	0% (0/36)	—	—
	n727	100% (55/55)	0% (0/51)	—	—
	n360	100% (*n* > 100)	0% (0/28)	—	—
	ma135	100% (*n* > 100)	0% (0/39)	—	—
	n179ts 20 °c	0% (*n* > 100)	0% (*n* > 100)	—	—
lin-4(e912), lin-14(n179ts) 20 °C		—	—	100% (9/9)	23% (8/35)
lin-28	*n1119*	100% (33/33)	3%† (1/31)	—	—
	n719	100% (*n* > 100)	8%† (3/37)	—	—
lin-29	*n333*	—	—	100% (*n* > 100)	100% (55/55)
	n1440	—	—	100% (*n* > 100)	100% (*n* > 100)

During continuous development, loss-of-function (*lf*) mutations of *lin-14* and *lin-28* cause the L/A switch to occur precociously (refs 3, 10; Fig. 1*a*). 'Precocious' L/A switch refers to the formation of adult lateral alae and absence of seam cell division at the L3 moult. In contrast, *lin-4(e912), lin-29(lf)* and the semi-dominant gain-of-function (*gf*) mutations of *lin-14(n536)* delay or completely inhibit the L/A switch (refs 3, 8, 9; Fig. 1*a*); their seam cells synthesize a larval cuticle and continue cell divisions at the L4 moult and they develop through supernumerary larval moults. 'Retarded' L/A switch refers to absence of lateral alae and occurrence of seam cell division at the L4 moult. The percentage of animals that express a precocious or retarded L/A switch is given. Where known precisely, number of animals examined is indicated in parentheses; '*n* > 100' is an estimation of the number of animals examined. — Corresponding mutant phenotype was never observed. Seam cell divisions were observed using Nomarski optics[18]. Adult lateral alae were visualized by side illumination under ×50 magnification in the dissecting microscope[3]. The *lin-14(n355n679ts)* (strain MT1388) was derived by reverting the semi-dominant gain-of-function *lin-14(n355)* mutation, and thus contains both the original semi-dominant mutation *n355* and the new temperature-sensitive intragenic suppressor *n679ts*. *lin-14(n355n679ts)* at 15 °C is semi-dominant and results in retarded defects. At 25 °C, *lin-14(n355n679ts)* exhibits precocious defects[9]. As severely retarded mutants such as *lin-14(n536)* and *lin-4(e912)* do not form dauer larvae, we used less retarded strains, *lin-14(n536/+)* and *lin-4(e912)*; *lin-14(n179ts)*. In the *lin-4(e912)*; *lin-14(n179ts)* double mutant at 20 °C, the retarded L/A switch is likely to be solely caused by the *lin-4(e912)* mutation, because *lin-14(n179ts)* animals at 20 °C are weakly precocious.

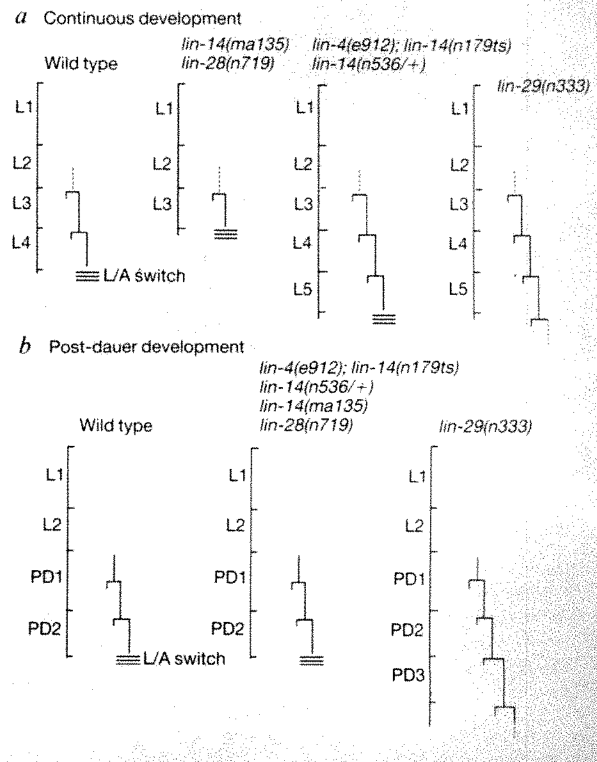
* Animals expressed the precocious or the retarded L/A switch in only local area(s) of the body after post-dauer development (see text).

† Occasionally, *lin-28* mutants displayed very small patches (corresponding to 1 or 2 seam cells) of precocious adult cuticle even after post-dauer development. This may result from the fact that some *lin-28* animals express patches of adult cuticle at the L2-to-dauer moult (data not shown), and thus these cells may have terminally differentiated before dauer larva formation.

hypodermal cell differentiation is regulated by separate temporal control systems, depending on the developmental history.

Dauer larvae were isolated from starved cultures by SDS selection[4], placed on food, and post-dauer development was examined. The morphological and behavioural defects that result from abnormal timing of the L/A switch in both retarded and precocious mutants of *lin-14*, *lin-4* and *lin-28* were efficiently suppressed in animals that developed from dauer larvae (Table 1). Cell-lineage analysis revealed that the precocious or retarded L/A switch defects were suppressed by the expression of a wild-type post-dauer developmental programme consisting of two larval stages (PD1 and PD2, analogous to L3 and L4) with the L/A switch at the PD2-to-adult moult (Fig. 1*b*). Suppression absolutely required dauer larva formation: mutant animals that

FIG. 1 Lateral hypodermal cell lineages during continuous and post-dauer development of wild type and heterochronic mutants. The vertical axis indicates the four post-embryonic larval stages, with horizontal marks indicating the times of hatching and each larval moult. L/A switch is indicated by triple bars. Cell lineages were followed in living nematodes at 20 °C using Nomarski optics[18]. *a*, Representative lateral hypodermal cell lineages, beginning at the L2 moult of continuous development (previous cell divisions of this lineage are not shown) of the wild type, two precocious mutants (*lin-14(ma135)* and *lin-28(n719)*) and three retarded mutants (*lin-4(e912)*; *lin-14(n179ts)*, *lin-14(n536/+)* and *lin-29(n333)*)[3]. *b*, Representative lateral hypodermal cell lineages during post-dauer development in precocious and retarded mutants beginning at the time of feeding developmentally arrested dauer larvae. PD1 and PD2 refer to the two post-dauer larval stages. Dauer larva arrest occurred at the second (L2) moult in all these animals. The diagrams are composites of lineages observed in several animals of each genotype. Some post-dauer lineages were followed intermittently[10]; the results of intermittent observation of multiple animals were confirmed by continuous observation of one or more individuals.



did not become dauer larvae in starved and crowded cultures were not suppressed, but dauer larvae from the same cultures were all suppressed. Suppression was not a function of time spent in dauer larva arrest, and dauer larvae induced by growth in the presence of the dauer-promoting pheromone[6], by starvation, or by the *ts* dauer-constitutive mutations[11] were equally suppressed. Finally, the mechanism of suppression does not result from heritable suppressor mutations or a restoration of *lin-4*, *lin-28* or *lin-14* gene activity. Presumptive null alleles, such as *lin-14(ma135)* and *lin-28(n719)* (ref. 10), show essentially full suppression. Thus the post-dauer developmental programme does not require *lin-4*, *lin-14* or *lin-28*.

The *lin-4* and *lin-14* mutants show an essentially wild-type post-dauer developmental programme even when they begin post-dauer development at abnormal stages. Although wild-type animals form dauer larvae only at the L2 moult, certain heterochronic mutants sometimes arrest as dauer larvae at abnormal stages—at the L1 moult in precocious *lin-14* mutants, or at the L3 moult in retarded *lin-14* and *lin-4* mutants[12]. The L1 or L3 dauer larvae execute an essentially normal post-dauer pathway, consisting of two larval stages and two rounds of lateral hypodermal cell division, with the L/A switch at the end of the PD2 stage (data not shown). Thus the post-dauer programme is essentially independent of events before dauer larva arrest. Interestingly, the PD1 stage was lengthened by 10–15 h (about the length of one larval stage) for L1 dauer larvae and was shortened by about 5–6 h for L3 dauer larvae. This suggests that the PD1 may be naturally flexible in length to allow for the resynchronization of development between the hypodermis and other tissues.

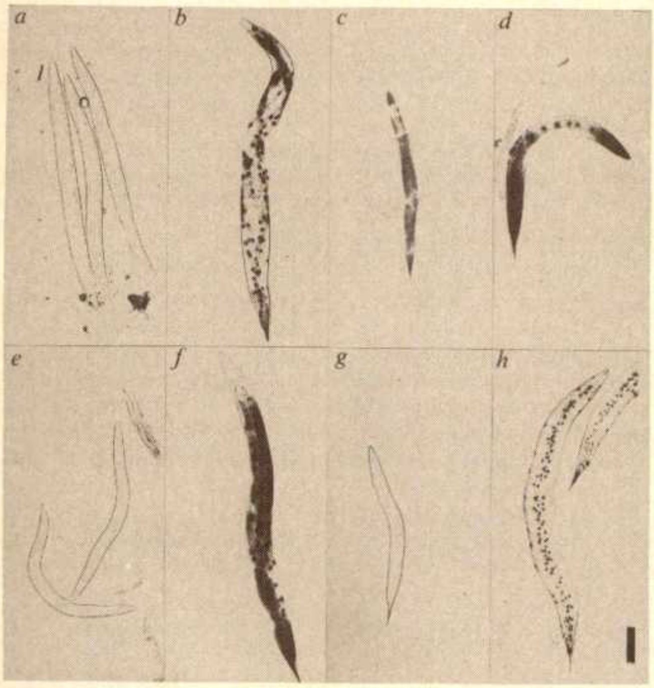


FIG. 2 Expression of a *col-19–β-gal* chimaeric gene in the wild type and *lin-14(n179ts)* precious mutants during continuous and post-dauer development. Top panel, animals that developed through continuous development: *a*, wild-type L4 larvae; *b*, wild-type adult; *c*, *lin-14(n179ts)* young L4 larva at 25 °C; *d*, *lin-14(n179ts)* adult at 25 °C. Bottom panel, animals that developed through post-dauer development: *e*, wild-type L4 larvae; *f*, wild-type adult; *g*, *lin-14(n179ts)* young L4 larva at 25 °C; *h*, *lin-14(n179ts)* adult at 25 °C. Animals were from strains of the indicated genotype stably transformed with the *col-19–β-gal* gene and a dominant marker *rol-6(su1006)* (ref. 19; C. Mello and V.A., unpublished) by microinjection of DNA into the hermaphrodite gonad. The *col-19–β-gal* chimaeric gene was a translational fusion between *col-19* and *β*-galactosidase gene in vector 22.04 (ref. 20; Z.L. and V.A., unpublished). As the *col-19–β-gal* fusion contains only 7 amino acids of the *col-19* gene, the adult-specific *col-19–β-gal* expression probably reflects adult-specific transcriptional initiation. *β*-Galactosidase activity in transgenic worms was assayed by staining fixed worms with a solution containing X-gal (5-bromo-4-chloro-3-indolyl-D-galactoside) (A. Fire and J. Shaw, personal communication). The *col-19–β-gal* fusion has a nuclear localization signal[20] that causes most of the staining to be localized to hypodermal nuclei, although in more intensely stained animals some of the staining apparently leaked from the nuclei into other areas of the body. Scale bar, 100 μm.

Generally, post-dauer development leads to complete suppression of heterochronic L/A switch defects (Table 1). However, certain mutants with elevated *lin-14* activity (*lin-14(gf)* or *lin-4(e912)* (ref. 10)) that display retarded defects after continuous development also display a residual retarded L/A-switch defect after post-dauer development (Table 1). In animals whose post-dauer development was followed using Nomarski optics, we found that this residual retarded defect in patches of cells resulted from 'incomplete dauer larva morphogenesis'[12] in the corresponding cell lineages. This indicates that expression of the post-dauer programme is variably blocked by elevated *lin-14* activity, and can occur cell-intrinsically. In contrast to these retarded mutants, essentially all precocious mutant animals were completely suppressed, although precocious mutants readily form incomplete dauer larvae (ref. 12, and Table 1). This indicates that expression of the post-dauer developmental programme in a particular hypodermal lineage is not sensitive to reduced *lin-14* or *lin-28* activity and does not require prior dauer larva differentiation.

The *lin-29* gene is unique among the heterochronic genes examined so far in that its loss causes a complete block in the execution of the L/A switch during post-dauer development as well as in continuous development (Table 1; Fig. 1). The *lin-29* gene is thought to be a direct activator of the L/A switch, and *lin-4*, *lin-14* and *lin-28* to regulate the stage-specific activation of *lin-29* (ref. 10). Our observations that post-dauer development suppresses the L/A switch defects of *lin-4*, *lin-14* and *lin-28* mutants but not of *lin-29* mutants suggests that *lin-29* is critical for both continuous and post-dauer development, but is subject to modes of regulation specific to each pathway. The only genes known to affect recovery from the dauer larva stage are *da*uer *f*ormation genes (*daf*) that also control entry into the dauer larva stage[11], but none of the *daf* mutants that we have examined display defects in the timing of the L/A switch during post-dauer or continuous development (data not shown).

Certain stage-specific collagen genes are regulated by *lin-29* during continuous and post-dauer development (Z.L. and V.A., unpublished). We tested whether post-dauer development restores the proper temporal specificity of these genes in heterochronic mutants. A *col-19–β-gal* chimaeric gene that is expressed adult-specifically in wild type during both developmental pathways (Fig. 2*a*, *b*, *e*, *f*; refs 13, 14) is expressed precociously in *lin-14(n179ts)* animals that develop continuously at the restrictive temperature (Fig. 2*c*, *d*). This precocious expression is corrected by post-dauer development (Fig. 2*g*, *h*). Thus post-dauer suppression of defects in the stage-specificity of cuticle morphogenesis reflects a restoration of the proper stage-specific transcription of *col-19* and perhaps of other genes regulated by *lin-29*. Although *lin-29* encodes a putative transcription factor (A. Rougvie and V.A. personal communication), we do not know if the *lin-29* product regulates *col-19* directly or indirectly.

Certain *C. elegans* mutants defective in cuticle structure or vulva-cell lineage exhibit a weaker or modified phenotype after post-dauer development[15,16]. These cases of post-dauer partial suppression or modification of phenotype differ quantitatively and qualitatively from the suppression of heterochronic mutants. The suppression is not as efficient as for the heterochronic mutants, and among vulva-cell specification and cuticle structure genes, post-dauer suppression seems to be the exception. In contrast, the entire regulatory pathway consisting of *lin-4*, *lin-14* and *lin-28* is completely dispensable for post-dauer development. These differences may reflect particularly acute constraints imposed on temporal control systems (as opposed to spatial patterning or cuticle morphogenesis) by the suspension of development at dauer larva arrest.

What mechanistic or developmental constraints account for the existence of separate temporal signals for continuous and post-dauer development? Heterochronic genes may encode components of a temporally dynamic system of interacting gene products. The *lin-14* activity must decrease between L1 and L2 to specify the proper timing and sequence of diverse events, including the L/A switch[9,10,17]; *lin-4* and *lin-28*, which interact with *lin-14*, might also experience precise temporal changes. The indefinite period of developmental arrest in the dauer larva stage would inevitably interrupt the dynamics of this system, and thus render *lin-4*, *lin-14* and *lin-28* useless for conveying temporal information to cells during post-dauer development. Other organisms with optional developmentally arrested stages, such as insects that undergo diapause, may use a similar strategy to cope with the temporal control problems necessarily associated with alternative developmental histories. ☐

Received 5 December; accepted 27 December 1990.

1. Alberch, P., Gould, S. J., Oster, G. F. & Wake, D. B. *Paleobiology* **5,** 296–317 (1979).
2. Gould, S. J. *Ontogeny and Phylogeny* (Harvard University Press, Cambridge, Massachusetts, 1977).
3. Ambros, V. & Horvitz, H. R. *Science* **226,** 409–416 (1984).
4. Cassada, R. C. & Russell, R. L. *Devl Biol.* **46,** 326–342 (1975).
5. Evans, A. A. F. & Perry, R. M. in *The Organization of Nematodes* (ed. Croll, N. A.) (Academic, New York, 1976).
6. Golden, J. M. & Riddle, D. L. *Science* **218,** 578–580 (1987).
7. Riddle, D. L. in *The Nematode* Caenorhabditis elegans (eds Wood, W. B. and the community of *C. elegans* researchers) (Cold Spring Harbor Laboratory, New York, 1988).
8. Chalfie, M., Horvitz, R. H. & Sulston, J. E. *Cell* **24,** 59–69 (1981).
9. Ambros, V. & Horvitz, H. R. *Genes Dev.* **1,** 398–414 (1987).
10. Ambros, V. *Cell* **57,** 49–57 (1989).
11. Riddle, D. L., Swanson, M. M. & Alberts, P. S. *Nature* **290,** 668–671 (1981).
12. Liu, Z. & Ambros, V. *Genes Dev.* **3,** 2039–2049 (1989).
13. Cox, G. N., Fields, C., Kramer, J. M., Rosenzweig, B. & Hirsh, D. *Gene* **76,** 331–344 (1989).
14. Cox, G. N. & Hirsh, D. *Molec. cell. Biol.* **5,** 363–372 (1985).
15. Ferguson, E. & Horvitz, H. R. *Genetics* **110,** 17–72 (1985).
16. Cox, G. N., Laufer, J. S. Kusch, M. & Edgar, R. *Genetics* **95,** 317–339 (1980).
17. Ruvkun, G. & Guisto, J. *Nature* **338,** 313–319 (1989).
18. Sulston, J. E. & Horvitz, R. H. *Devl Biol.* **56,** 110–156 (1977).
19. Kramer, J. M., French, R. P., Park, E. & Johnson, J. J. *Molec. cell. Biol.* **10** (1990).
20. Fire, A., Harrison, S. & Dixon, D. *Gene* **93,** 189–198 (1990).

ACKNOWLEDGEMENTS. We thank S. Euling, R. Feinbaum, G. Struhl, D. Levitan, C. Mello, A. Rougvie and other members of the laboratory for critical reading of the manuscript, and P. Cherbas for comments.

Function of DnaJ and DnaK as chaperones in origin-specific DNA binding by RepA

Sue Wickner*, Joel Hoskins† & Keith McKenney†

* Laboratory of Molecular Biology, National Cancer Institute,
National Institutes of Health, Bethesda, Maryland 20892, USA
† Center for Advanced Research in Biotechnology, National Institute of
Standards and Technology, Rockville, Maryland 20850, USA

HEAT-shock proteins are normal constituents of cells whose synthesis is increased on exposure to various forms of stress. They are interesting because of their ubiquity and high conservation during evolution. Two families of heat-shock proteins, hsp60s and hsp70s, have been implicated in accelerating protein folding and oligomerization and also in maintaining proteins in an unfolded state, thus facilitating membrane transport[1–5]. The *Escherichia coli* hsp70 analogue, DnaK, and two other heat-shock proteins, DnaJ and GrpE, are required for cell viability at high temperatures and are involved in DNA replication of phage λ and plasmids P1 and F[6–10]. These three proteins are involved in replication *in vitro* of P1 DNA along with many host replication proteins and the P1 RepA initiator protein[11,12]. RepA exists in a stable protein complex with DnaJ containing a dimer each of RepA and DnaJ[11]. We report here that DnaK and DnaJ mediate an alteration in the P1 initiator protein, rendering it much more active for *ori*P1 DNA binding.

TABLE 1 Temperature requirement for activation of RepA

Incubation at 24 °C (15 min)	Incubation at 0 °C (15 min)	*ori*P1 DNA bound (%)
Complete	No additions	26
No additions	Complete	<2
Minus ATP	+ATP	<2
Minus *ori*P1 DNA	+*ori*P1 DNA	32
Minus DnaJ	+DnaJ	4
Minus DnaK	+DnaK	4
Minus RepA	+RepA	3

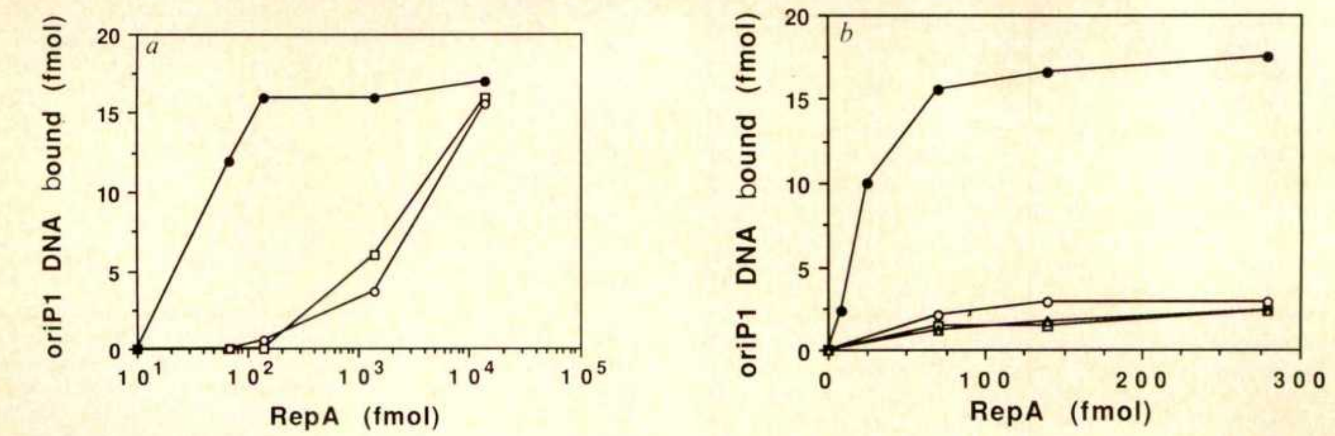
DNA binding was measured by retention on nitrocellulose filters. Reaction mixtures for the first incubation at 24 °C contained the components described in Fig. 1 with the omissions indicated and with 20 ng RepA, 30 ng DnaJ and 100 ng DnaK. After 15 min, the reactions were chilled on ice, the omitted components were added, and the reactions were continued for 15 min at 0 °C.

RepA binds specifically to five 19-base-pair (bp) direct repeats in the P1 origin[13]. We observed that the DnaJ–RepA protein complex also binds to the P1 origin (Fig. 1*a*). Surprisingly, about a 100-fold molar excess of RepA dimers to binding sites was required for binding by RepA alone or in a complex with DnaJ (Fig. 1*a*). These results suggested that the preparations of RepA and DnaJ–RepA complex were largely inactive or the affinity of RepA for DNA was very low.

Because it seemed likely that the other heat-shock proteins may function with DnaJ, we added DnaJ, DnaK and GrpE to DNA-binding reactions with RepA. About 100-fold less RepA was required for *ori*P1 DNA binding when DnaJ, DnaK and ATP were added to the reaction mixtures (Fig. 1*a–c*). GrpE was not required for RepA activation (Fig. 1*c*). DnaJ–RepA protein complexes could be activated by DnaK and ATP, without additional DnaJ. ATP hydrolysis is probably required because the non-hydrolysable ATP analogue ATP-γ-S was inactive and was a competitive inhibitor of the reaction.

The stimulation by DnaJ, DnaK and ATP of *ori*P1 DNA binding by RepA depended both on the time of incubation and the temperature. DNA binding increased linearly at 24 °C for 20 min, but was barely detectable when the reactions were carried out at 0 °C (Fig. 2). Because protein–DNA binding is expected to occur at 0 °C, we attempted to divide the DnaJ- and DnaK-stimulated binding reaction. In a first reaction at 24 °C, we incubated all five components (DnaJ, DnaK, RepA, ATP and *ori*P1 DNA) or four of them. Then in a second incubation at 0 °C, we added the omitted component and measured *ori*P1 DNA bound (Table 1). DnaK, DnaJ, RepA and ATP were each required for DNA binding in the first incubation, but *ori*P1 DNA was not. The first reaction in the absence of DNA proceeded linearly for 20 min at 24 °C, whereas the DNA-binding reaction was complete by 30 s at 0 °C (Fig. 2). The same requirements were necessary when the first reaction was carried out at 30 °C or 42 °C. When DnaJ and RepA were replaced by the DnaJ–RepA complex, the activation reaction still required a 20-min incubation at 24 °C with DnaK and ATP. This is consistent with our previous observation that the DnaJ–RepA complex forms rapidly at 0 °C on mixing the two proteins[11]. These experiments show that DnaJ and DnaK, in an ATP and temperature-dependent reaction, activate RepA in the absence of DNA so that it can then rapidly bind to *ori*P1 DNA.

Of the three proteins required for the reaction, only RepA ends up bound to the P1 DNA. We prepared a large reaction mixture, isolated the protein–DNA complex by gel filtration, subjected the material to SDS-PAGE, and stained for protein. Densitometer analysis of the gel showed that RepA was the only protein detectable (Fig. 3). Protein–DNA complexes similarly



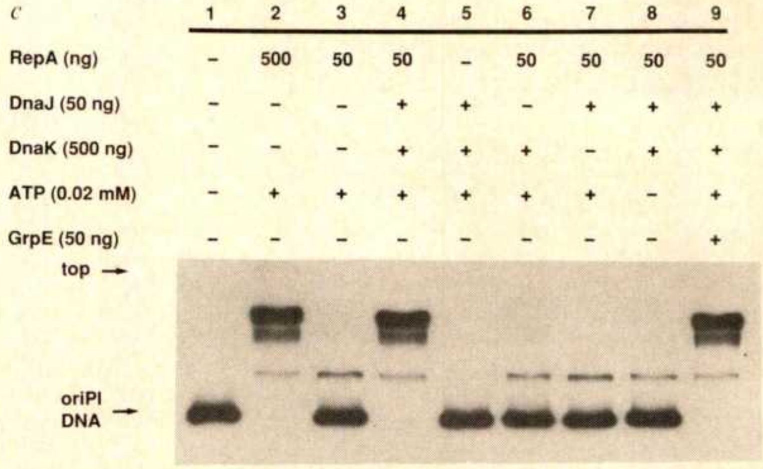

c
1
2
3
4
5
6
7
8
9
RepA (ng) – 500 50 50 – 50 50 50 50
DnaJ (50 ng) – – – + + – + + +
DnaK (500 ng) – – – + + + – + +
ATP (0.02 mM) – + + + + + + – +
GrpE (50 ng) – – – – – – – – +
top
oriPl DNA

FIG. 1 *a*, *ori*P1 DNA binding by RepA alone (○), DnaJ–RepA complex alone (□) and RepA in combination with 30 ng DnaJ, 100 ng DnaK and ATP (●). The molar amount of RepA is calculated from the observations that RepA is a dimer in solution[11,13] and the DnaJ–RepA complex contains a dimer each of RepA and DnaJ[11]. *b*, Requirements for activation of RepA for *ori*P1 DNA binding. ●, Complete reactions containing RepA, 30 ng DnaJ and 100 ng DnaK; reactions lacking ATP (○), DnaK (□) or DnaJ(△). *c*, *ori*P1 DNA binding as measured by gel retardation. DNA binding reactions were carried out as described for above but with 25 μM ATP, 5 μg ml^{-1} calf thymus DNA and proteins in amounts indicated. After 20 min at 24 °C, reactions were analysed by electrophoresis on 5% acrylamide gels[13]. Intermediate shifted bands were generated by subsaturating amounts of RepA[13].

METHODS. Reaction mixtures (20 μl) contained 20 mM Tris–HCl, pH 7.5, 40 mM KCl, 100 mM NaCl, 10 mM $MgCl_2$, 0.1 mM EDTA, 1 mM dithiothreitol, 50 μM ATP, 50 μg ml^{-1} BSA, 50 μg ml^{-1} calf thymus DNA, 5 ng labelled *ori*P1 DNA (285-bp fragment containing P1 origin DNA from position 381 to 611[16]), DnaK, DnaJ and RepA. Purified DnaK, DnaJ, GrpE and RepA proteins[11] were more than 95% pure as determined by SDS-PAGE; DnaJ–RepA complex isolated from cell extracts[11] was 80% pure. Incubations were for 20 min at 24 °C. Reaction mixtures were then filtered through nitrocellulose filters and filter-bound radioactivity was measured.

isolated from reaction mixtures lacking either DnaJ or ATP contained no detectable proteins. Thus, DnaJ and DnaK activate RepA to bind to *ori*P1 DNA but are not part of the protein–DNA complex.

The molecular change in RepA brought about by DnaJ and DnaK is not so far understood. Activation does not seem to involve proteolytic processing, because activated RepA isolated from DNA or from reaction mixtures comigrated with purified RepA on SDS-PAGE. DnaK autophosphorylates[14], but there was no detectable phosphorylation of RepA during the activation reaction. DnaK and other hsp70s facilitate disaggregation[4,5], but RepA does not appear to aggregate as measured by gel filtration of reaction mixtures containing RepA alone or RepA plus DnaJ and DnaK but not ATP. DnaJ and DnaK could dissociate RepA dimers to monomers or cause some subtle conformational change in RepA.

DnaJ, DnaK and GrpE may also function *in vivo* to activate DNA binding by RepA. Repression of the autoregulated *repA* promoter by RepA is 2- to 10-fold less in *dnaJ*, *dnaK* and *grpE* mutant strains compared to wild-type strains[15]. This could be explained by our finding that DnaJ and DnaK activate the DNA-binding function of RepA. And what is the biochemical function of GrpE? We have demonstrated that it is required for P1 replication *in vitro* but that it is not required for the activation of RepA.

A model for RepA activation is shown in Fig. 4. The inactive

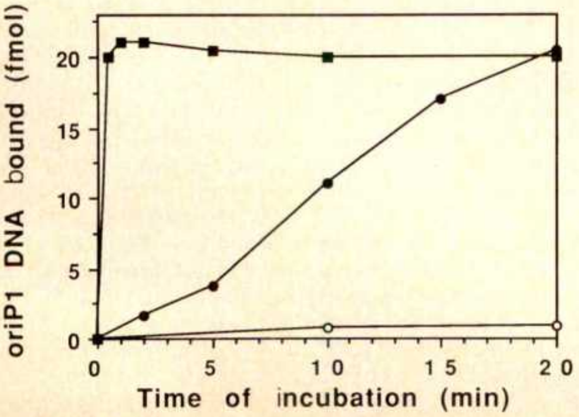


FIG. 2 Time course of RepA activation. DNA binding was measured by retention on nitrocellulose filters as described in Fig. 1 with 30 ng RepA, 30 ng DnaJ and 200 ng DnaK. Reactions were incubated for the times indicated at 0 °C (○) or at 24 °C (●). Other reactions were preincubated for 20 min at 24 °C in the absence of DNA. Then *ori*P1 DNA was added and the reactions were continued for the times indicated at 0 °C (■).

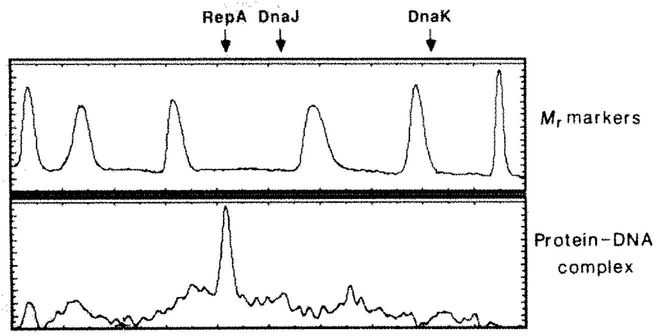


FIG. 3 Determination of proteins associated with *ori*P1 DNA. The reaction was as described in Fig. 1, but in 2 ml containing 5 μg RepA, 5 μg, DnaJ, 50 μg DnaK, 500 ng $[^3H]$*ori*P1 DNA (1,182-bp DNA fragment) and 5 $\mu g\ ml^{-1}$ calf thymus DNA; BSA was omitted. After 20 min at 24 °C, the reaction was applied to a 1×25 cm Sepharose 6B column equilibrated with 20 mM Tris–HCl, pH 7.5, 10 mM $MgCl_2$, 1 mM dithiothreitol, 0.1 mM EDTA, 40 mM KCl and 100 mM NaCl. The column was eluted with the same buffer. Fractions in the void volume (containing radioactive *ori*P1 DNA and associated proteins) were concentrated by precipitation with 15% tricarboxylic acid. The pellet was washed with acetone, dissolved in SDS sample buffer, analysed by 10–15% SDS-PAGE, stained with Coomassie blue, and scanned with a densitometer. Molecular weight markers were BioRad low-range SDS-PAGE standards.

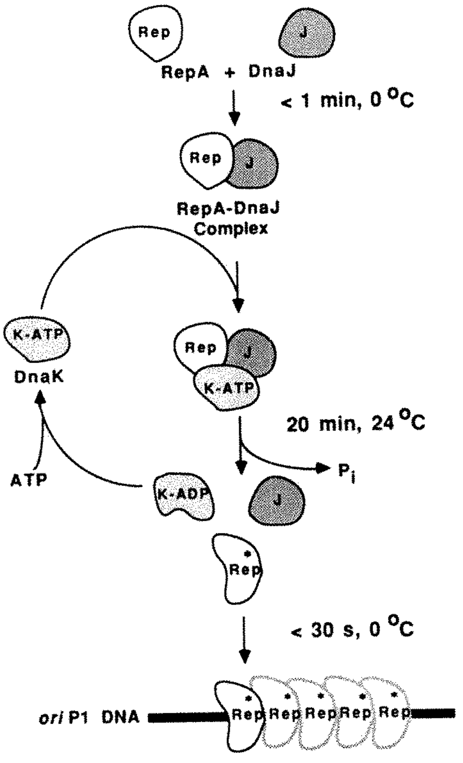


FIG. 4 Model for activation of RepA.

form of RepA binds tightly and rapidly to DnaJ at 0 °C to form a 2 : 2 tetramer[11]. DnaJ may be as a protein cofactor that targets RepA for subsequent recognition and activation by DnaK in an ATP and temperature-dependent reaction. The energy of ATP hydrolysis is probably used to activate RepA and to release DnaJ. The activated RepA then can bind rapidly to DNA at 0 °C. This activation may be involved in the regulation of the rate of initiation of DNA replication and the regulation of expression of the autoregulated *repA* gene.

To our knowledge, this is the first demonstration in an *in vitro* system that DnaJ and DnaK function as molecular chaperones to activate a sequence-specific DNA binding protein. Because the heat-shock proteins are so highly conserved between bacteria and eukcaryotes, it is likely that similar mechanisms operate to regulate gene expression and replication in other systems. ☐

Received 16 November 1990; accepted 10 January 1991.

1. Rothman, J. E. *Cell* **59**, 591–601 (1989).
2. Neidhardt, F. & VanBogelen, R. in Escherichia coli *and* Salmonella typhimurium (eds Neidhardt, F. *et al.*) 1334–1345 (American Society for Microbiology, Washington, DC, 1987).
3. Lindquist, S. & Craig, E. A. *A. Rev. Genet.* **22**, 631–677 (1988).
4. Pelham, H. R. B. in *Stress Proteins in Biology and Medicine* (eds Morimoto, R., Tissieres, A. & Georgopoulos, C.) 287–299 (Cold Spring Harbor Laboratory Press, New York, 1990).
5. Morimoto, R. I., Tissieres, A. & Georgopoulos, C. in *Stress Proteins in Biology and Medicine* (eds Morimoto, R., Tissieres, A. & Georgopoulos, C.) 1–36 (Cold Spring Harbor Laboratory Press, New York, 1990).
6. Georgopoulos, C., Ang, D., Liberek, K. & Zylicz, M. in *Stress Proteins in Biology and Medicine* (eds Morimoto, R., Tissieres, A. & Georgopoulos, C.) 191–221 (Cold Spring Harbor Laboratory Press, New York, 1990).
7. Bukau, B. & Walker, G. *J. Bact.* **171**, 6030–6038 (1989).
8. Tilly, K. & Yarmolinsky, M. *J. Bact.* **171**, 6025–6029 (1989).
9. Kawasaki, Y., Wada, C. & Yura, T. *Molec. gen. Genet.* **220**, 277–282 (1990).
10. Ezaki, B., Ogura, T., Mori, H., Niki, H. & Hiraga, S. *Molec. gen. Genet.* **218**, 183–189 (1989).
11. Wickner, S. *Proc. natn. Acad. Sci. U.S.A.* **87**, 2690–2695 (1990).
12. Wickner, S. & Chattoraj, D. *Proc. natn. Acad. Sci. U.S.A.* **84**, 3668–3672 (1987).
13. Abeles, A. L. *J. biol. Chem.* **261**, 3548–3555 (1986).
14. Zylicz, M., LeBowitz, J., McMacken, R. & Georgopoulos, C. *Proc. natn. Acad. Sci. U.S.A.* **80**, 6431–6435 (1983).
15. Tilly, K., Sozhamannan, S. & Yarmolinsky, M. *The New Biologist* **2**, 1–6 (1990).
16. Abeles, A. L., Snyder, K. M. & Chattoraj, D. K. *J. molec. Biol.* **173**, 307–324 (1984).

ACKNOWLEDGEMENTS. We thank Nic Cozzarelli and Mike Maurizi for their helpful discussions and comments on this manuscript.

Structural architecture of an outer membrane channel as determined by electron crystallography

B. K. Jap, P. J. Walian & K. Gehring

Donner Laboratory, Lawrence Berkeley Laboratory, University of California, Berkeley, California 94720, USA

PORINS are a family of membrane channels commonly found in the outer membranes of Gram-negative bacteria where they serve as diffusional pathways for waste products, nutrients and antibiotics, and can also be receptors for bacteriophages[1,2]. Porin channels have been shown *in vitro* to be voltage-gated[3–6]. They can exhibit slight selectivities for certain solutes; for example PhoE porin has some selectivity for anionic and phosphate-containing compounds[1,7]. Unlike many known membrane proteins which often contain long stretches of hydrophobic segments that are believed to traverse the membrane in a helical conformation, porins are found to have charged residues distributed almost uniformly along their primary sequences and have most of their secondary structure in a β-sheet conformation[8–10]. We have made crystalline patches of PhoE porin embedded in a lipid bilayer and have used these to determine the structure of PhoE porin by electron crystallography to a resolution of 6Å. The basic structure consists of a trimer of elliptically shaped, cylindrical walls of β sheet. Each cylinder has an inner lining, formed by parts of the polypeptide, that defines the channel size. The structure provides a clue as to how deletions of segments of polypeptide, which are found in certain mutants, can result in an actual increase in the channel size.

We have reconstituted purified PhoE porin with phospholipids to form highly coherent, crystalline membrane patches that diffract to a resolution better than 3.0 Å (ref. 11). The crystalline membrane patches have a space group $P2_12_12$ ($a = 150$ Å and $b = 129$ Å). A high-resolution projection map[12] and a three-dimensional (3-D) electron diffraction data set[11] have been obtained. The predominant features seen in the projection

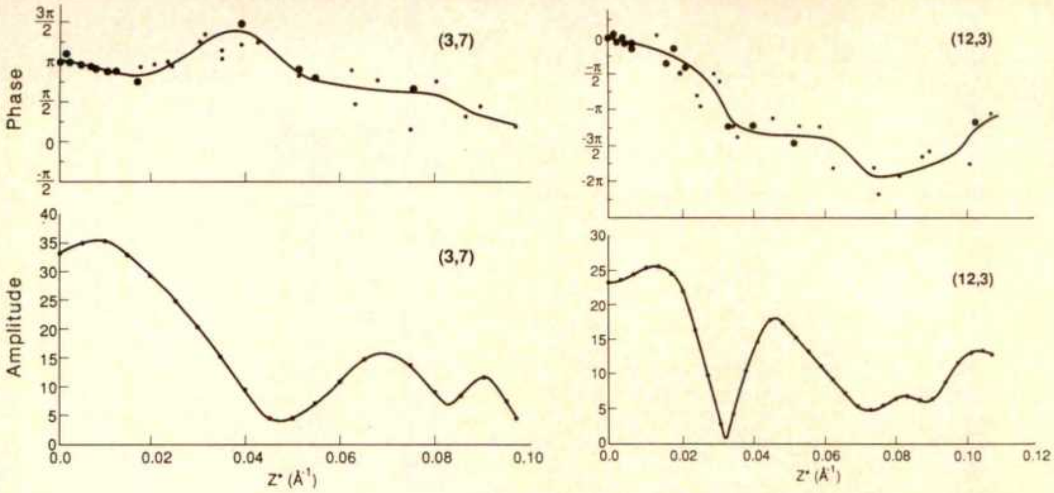


FIG. 1 Amplitudes and phases along two lattice lines together with fitted curves. The smooth curves were obtained by weighted least-squares fitting as described by Agard[18]. The phase measurement is plotted with two different sizes of circles, a large circle (IQ 1 to 4)[16] or a small circle (IQ 5 to 8), to indicate the reliability of the phase measurement. The amplitude measurements are derived from the earlier diffraction data. The fitted curves of phase and amplitude were used to generate the 3-D map.

map are high density, elliptical, ring-like structures which have been interpreted as the projected images of β sheet, seen edge-on. Each ring-like structure consists of 'beads', some of which have been interpreted as an end-on view of β strands. This interpretation is consistent with the diffraction data indicating that within PhoE porin a significant fraction of the β strands are oriented perpendicular to the membrane plane, whereas the predominant population of β strands are tilted by an average of 35° from the membrane normal[11]. At the threefold symmetry axis of the trimer, there is a very-low-density zone that has been proposed as the location of lipopolysaccharide in the native membrane.

Electron diffraction patterns and images of PhoE porin crystals, embedded in trehalose and maintained at about −120 °C, were recorded from samples tilted from 0 to about 60°, using low-dose recording techniques. Both conventional flood beam illumination and spot-scan imaging techniques[13,14] have been used. Images that were found to yield sharp, high-resolution spots by optical diffraction were selected, and digitized with a Perkin Elmer Model 1010M scanning densitometer. Images of tilted specimens were scanned with a 10-μm square aperture and a step size of 10 μm in arrays of 3,168 by 3,168 pixels. Twenty images at tilt angles ranging from 20° to about 60° were digitized and processed. The tilt axes and angles of images were determined as described by Shaw and Hills[15]. Computer processing of the digitized images followed the methods of Henderson *et al.*[16], mainly using computer software from the MRC (Laboratory of Molecular Biology, Cambridge, UK). The processing included corrections of lattice distortions in the image, the effect of the contrast transfer function, and the defocus gradient of

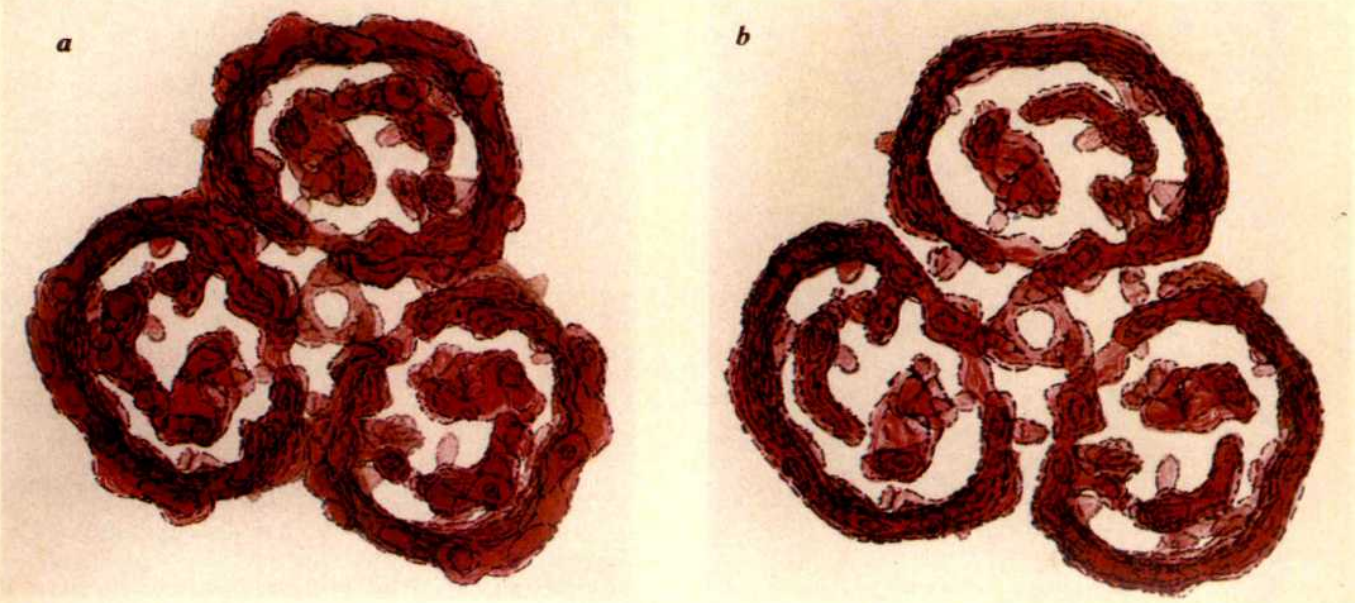


FIG. 2 Views of stacked serial sections obtained from the three-fold symmetrized 3-D map. Only the positive contour line (broken line) is shown here, representing the protein domains. The density in the map is contoured at an interval of 1/5 of the maximum density, starting at a level of 20%. The protein domains are also highlighted in red to enhance the 3-D perspective. *a*, The stacked 3-D model of thickness 36 Å viewed from the extracellular side of the structure. *b*, The bottom half of the structure. The maps show that the 3-D density consists of three elliptical, cylindrical walls that are arranged into a trimer. The elliptical cylinders have a major axis of ~38 Å and a minor axis of ~28 Å as measured from the centre of the wall. The cylinder wall extends to about 45 Å in length; however, beyond about 35 Å, the map is somewhat noisy. Each cylinder wall is clearly defined and there is no indication that the three channels of a trimer merge into a single pore. Within each cylinder, the substructure, also in our projection map[12], appears to extend along the entire length of the channel; the channel is not straight and is located off the elliptical cylinder axis. At the threefold symmetry axis, there is a low-density region that gradually decreases in size as it traverses from the extracellular to the periplasmic domain, reaching the smallest diameter about two-thirds of the way through the membrane. This region has previously been proposed to contain lipopolysaccharide on the extracellular side.

the tilted images[16]. The tilted images were brought to a common phase origin as described previously[17]. This was done by minimizing the difference between the phases of Fourier components of a given tilted image and the phases along the corresponding lattice lines determined previously. Initially, images with small tilt angles were merged with the projection map in which the phase values have been set to the nearest values of 0 or π, and then images with increasingly larger tilt angles were sequentially combined with the merged data. The phase errors resulting from such merging are less than 25° for each image to a resolution of 6 Å.

The 3-D map was reconstructed by Fourier transformation of hybrid structure factors created from the phase information derived from the images and more accurate amplitudes obtained from the 3-D electron diffraction data[11]. The structure factors were derived from curves that were obtained from fitting the merged amplitude and phase data using the program LATLINE[18]. Fitting of curves to the data was performed with a weighted least-squares algorithm with a constraint that there is no electron density outside a 55 Å-thick envelope, surrounding the membrane. This width, somewhat wider than the lipid bilayer, was chosen to avoid the possibility of erroneous truncation of real structural density. The tilt axes and angles of all the images were further refined by minimizing the phase deviation between the experimental data and the smooth curves that were obtained from the initial curve fitting. These new tilt axes and angles were used to obtain the refined 3-D data set. Examples of plots of amplitude and phase and their fitted curves used in our 3-D reconstruction are shown in Fig. 1. In the current reconstruction, structure factors were truncated to a resolution of about 6 Å. As a result of not having data from specimens with tilt angles greater than 60 degrees, the effective resolution is about 6 Å parallel to the membrane plane and about 8 Å normal to the membrane.

The 3-D reconstruction of PhoE porin at 6 Å is shown in Fig. 2. The reconstruction shows several interesting features, which have previously been seen only in projection[12,17]. The PhoE 3-D map confirms that three high-density, elliptical, cylindrical walls of β sheet are arranged as a trimer, with their cylinder axes almost parallel to each other and to the threefold axis, which is perpendicular to the membrane. The elliptical wall measures $\sim$38 Å by $\sim$28 Å along the major and minor axies, respectively. The length of the cylinder wall is $\sim$45 Å, beyond $\sim$35 Å, the map is somewhat noisy. Each cylinder wall is clearly defined, and there is no indication that the three channels of a trimer merge into a single pore, confirming our earlier interpretation of the negatively stained sample map[17]. Within each cylinder, additional density is found to be predominantly situated in contact with the part of the wall that is furthest away from the threefold symmetry axis, and this density appears to extend across the entire length of the channel. The open part of the channel therefore appears to be located off the central axis of the elliptical cylinder[19], and the centre-to-centre distance between the apparent position of the aqueous channels is somewhat smaller than the distance between the centres of the trimeric, elliptical cylinders, which is about 45 Å. This is consistent with our negatively stained data showing that, within the trimer, the greatest centre-to-centre channel distance is about 35 Å. In our current map, it is rather difficult to define the channel shape clearly. The channel itself does not appear to run straight, which would be consistent with the negatively stained 3-D map, showing that the trimeric channels come close to each other as they traverse the membrane but they do not merge.

It is reasonable to believe that the additional density seen within each elliptical cylinder represents a region of the polypeptide that defines the channel size and is important in determining the channel selectivity. In fact, deletions of 6–15 residues in a specific region of the primary sequence, found in certain mutants, have been shown to greatly increase pore permeability[20,21]. Point mutations in this region have also been found to alter the pore permeability and selectivity. This suggests that this specific region of the sequence forms a part of the channel itself, and deletions in this segment would, therefore, result in significant increases in the channel size.

At the threefold symmetry axis of the trimer, there is a region of very low density that has been interpreted to be the site of lipopolysaccharide on the extracellular surface of the membrane in the native state. The presence of lipopolysaccharide at this region has also recently been reported by Hoenger *et al*[22]. The low-density region is somewhat narrower in the periplasmic half of the membrane, indicating a reduced amount of lipid at the threefold symmetry axis on the periplasmic side of the membrane.

Recently, porin from *Rhodopseudomonas capsulata* has been crystallized, yielding X-ray diffraction intensities beyond 3.2 Å (ref. 23). With the use of X-ray crystallographic methods, a 3-D structural map of this trimeric porin has also been obtained to a resolution of 6 Å (ref. 24) and very recently its partial model structure at 3.0 Å resolution[25], with about three-quarters of the protein mass accounted for, has also been reported. The 6 Å map of this porin is different from that of PhoE porin, and does not show the well-defined cylindrical β-sheet wall which is seen in our 3-D map of PhoE porin or in the projection maps of PhoE and OmpF porins[17,26]. The 3-D map of *R. capsulata* porin shows two possible channels per porin monomer: one having a diameter of about 6–10 Å and the other somewhat narrower, with a diameter of about 5 Å. The centre-to-centre separation of the larger *R. capsulata* channels is roughly 44 Å, which is somewhat greater than that of PhoE porin as derived from either our current 6 Å map or our previous negatively stained 3-D map. Interestingly, the structure of *R. capsulata* porin at 3.0 Å resolution does show some similarity to that of PhoE porin at 6 Å resolution. Both porins consist of elliptical, cylindrical walls of β sheet that are arranged into a trimer. There are also many differences. The cylindrical walls forming the monomer-monomer contact region of the trimer of *R. capsulata* is significantly shorter than the overall length of the whole cylinder. Therefore, the trimeric channels form a common chamber at one side of the channel. In contrast, each cylinder wall of PhoE porin is clearly defined in the region of monomer–monomer contact, and there is no indication that the three channels of a trimer merge into a single pore. Within each cylinder, the substructure of PhoE porin appears to extend across the entire length of the channel although that of *R. capsulata* porin is confined around the central region of the channel. Another significant difference between these two porins can be found in their oligomeric states. In contrast to other porins that normally form very stable trimers, *R. capsulata* porins have been found in monomeric form even under mild purification conditions. The question of the similarity between the architecture of these two porins will have to await the availability of their respective detailed molecular structures. □

Received 4 December 1990; accepted 4 January 1991.

1. Nikaido, H. & Vaara, M. *Microbiol. Rev.* **49,** 1–32 (1985).
2. Lugtenberg, B. & Van Alphen, L. *Biochim biophys. Acta* **737,** 51–115 (1983).
3. Schindler, H. & Rosenbusch, J. P. *Proc. natn. Acad. Sci. U.S.A.* **75,** 3751–3755 (1978).
4. Dargent, B., Hofmann, W., Pattus, F. & Rosenbusch, J. P. *EMBO J.* **5,** 773–778 (1986).
5. Xu, G., Shi, B., McGroarty, E. J. & Tien, H. T. *Biochim. biophys. Acta* **862,** 57–64 (1986).
6. Mauro, A., Blake, M. & Labarca, P. *Proc. natn. Acad. Sci. U.S.A.* **85,** 1071–1075 (1988).
7. Korteland, J., Tommassen, J. & Lugtenberg, B. *Biochim biophys. Acta* **690,** 282–289 (1982).
8. Rosenbusch, J. P. *J. biol. Chem.* **249,** 8019–8029 (1974).
9. Kleffel, B., Garavito, R. M., Baumeister, W. & Rosenbusch, J. P. *EMBO J.* **4,** 1589–1592 (1985).
10. Tommassen, J. *Membrane Biogenesis* NATO ASI Series Vol. 16 (ed. Op den Kamp J.A.F.) 351–373 (NATO 1988).
11. Walian, P. J. & Jap, B. K. *J. molec. Biol.* **215,** 429–438 (1990).
12. Jap, B. K., Downing, K. H. & Walian, P. J. *J. struct. Biol.* **103,** 57–63 (1990).
13. Downing, K. H. & Glaeser, R. M. *Ultramicroscopy* **20,** 269–278 (1986).
14. Bullough, P. & Henderson, R. *Ultramiscoscopy* **21,** 223–230 (1987).
15. Shaw, P. J. & Hills, G. J. *Micron* **12,** 279–282 (1981).
16. Henderson, R. *et al. J. molec. Biol.* **213,** 899–929 (1990).
17. Jap, B. K. *J. molec. Biol.* **205,** 407–419 (1989).
18. Agard, D. A. *J. molec. Biol.* **167,** 849–852 (1983).
19. Jap, B. K. & Walian, J. P. *Q. Rev. Biophys.* **23–4,** 367–403 (1990).
20. Misra, R. & Benson, S. A. *J. Bact.* **170,** 3611–3617 (1988).

21. Benson, S. A., Occi, J. L. L. & Sampson, B. A. *J. molec. Biol.* **203,** 961–970 (1988).
22. Hoenger, A., Gross, H., Aebi, U. & Engel, A. *J. struct. Biol.* **103,** 185–195 (1990).
23. Nestel, U. *et al. FEBS Lett.* **242,** 405–408 (1989).
24. Weiss, M. S. *et al. FEBS Lett.* **256,** 143–146 (1989).
25. Weiss, M. S., Wacker, T., Weckesser, J., Welte, W. & Schulz, G. E. *FEBS Lett.* **267,** 268–272 (1990).
26. Sass, H. J. *et al. J. molec. Biol.* **209,** 171–175 (1989).

ACKNOWLEDGEMENTS. We thank K. H. Downing and R. M. Glaeser for helpful discussions. This work is supported by the Office of Health and Environmental Research, US Department of Energy and the NIH.

A haemoprotein with kinase activity encoded by the oxygen sensor of *Rhizobium meliloti*

Marie A. Gilles-Gonzalez, Gary S. Ditta
& Donald R. Helinski

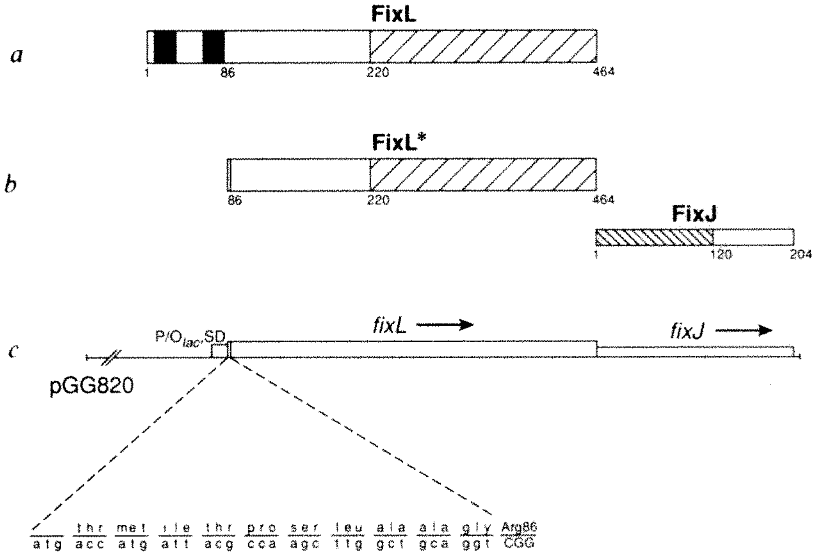
Department of Biology and Center for Molecular Genetics, 0634,
University of California, San Diego, La Jolla, California 92093, USA

THE expression of the nitrogen-fixation genes of *Rhizobium meliloti* is controlled by oxygen[1,2]. These genes are induced when the free oxygen concentration is reduced to microaerobic levels. Two regulator proteins, FixL and FixJ, initiate the oxygen-response cascade, and the genes that encode them have been cloned[2,3]. The *fixL* product seems to be a transmembrane sensor that modulates the activity of the *fixJ* product, a cytoplasmic regulator[3]. FixL and FixJ are homologous to a family of bacterial two-component regulators[4], for which the mode of signal transduction is phosphorylation (reviewed in refs 5–9). We report here the purification of both FixJ and a soluble truncated FixL (FixL*), overproduced from a single plasmid construct. FixL* catalyses its own phosphorylation and the transfer of the γ-phosphate of ATP to Fix J. The resulting FixJ–phosphate linkage is sensitive to base, as are the aspartyl phosphates of homologous systems. Visible spectra of purified FixL* show that it is an oxygen-binding haemoprotein. We propose that FixL senses oxygen through its haem moiety and transduces this signal by controlling the phosphorylation of FixJ.

Two long stretches of hydrophobic amino acids near the N terminus of FixL were deleted, to facilitate purification (Fig. 1*a* and *b*). This truncated form of FixL, designated FixL*, was coexpressed in *Escherichia coli* with FixJ, because FixL and FixJ might stabilize each other's activity. This was achieved by constructing a plasmid in which a 5′ truncated *fixLJ* operon was placed under the control of a *lac* promoter-operator (Fig. 1*c*). Two new proteins having relative molecular masses (M_rs) expected for either FixL* (M_r 42,803) or FixJ (M_r 22,849) were revealed on electrophoresis of whole-cell lysates from isopropyl-β-D-thiogalactoside (IPTG) induced, but not from non-induced, cells carrying the cloned genes. The presence of FixL* was confirmed by western immunoblot analysis using an antibody specific for the newly induced protein of $M_r \sim 43,000$ (43K). FixJ was the only 23K phosphoprotein that could be detected and was present exclusively in induced cells that carried the cloned genes. It also reacted to FixJ-specific antibody.

Both FixL* and FixJ partitioned to the soluble fraction of the sonicated cells (Fig. 2, lane a). They were purified more than 10-fold, as measured by protein assays using ion-exchange chromatography (Fig. 2, lane b). A peak of orange-red fractions was apparent. Gel electrophoresis and phosphorylation assays showed that these fractions contain both proteins. (On subsequent purification, the colour stayed with FixL*.) FixJ (>90% pure; about 10 mg from 20 g cells) was resolved from FixL* by gel filtration, indicating that they were not stably complexed (Fig. 2, lanes c and d). The native M_rs estimated from gel filtration were 50K for FixJ and 93K for FixL*, close to the values expected for dimeric FixJ (46K) and dimeric FixL* (86K). Orange-red FixL* of >95% purity (about 11 mg from 20 g cells) was obtained after further purification on MonoQ (Fig. 2, lane e).

Purified FixL* was reacted with [γ-^{32}P]ATP and examined by SDS-PAGE (Fig. 3*a*) and autoradiography (Fig. 3*b*). Radiolabel was detected in FixL*, indicating that FixL* can covalently attach to the γ phosphate of ATP (Fig. 3*a* and *b*, lane a). By contrast, no radiolabel was found in FixJ alone incubated under the same conditions (Fig. 3*a* and *b*, lane b). When the two proteins were incubated together with ATP, both FixL* and FixJ were labelled (Fig. 3*a* and *b*, lane c), indicating that FixL* is a kinase that can catalyse both its own phosphorylation and phosphorylation of Fix J. When the phosphorylated proteins were exposed to 0.5 M NaOH at 55 °C for 5 min, all the radiolabelled phosphate was hydrolysed from phospho-FixJ, whereas phospho-FixL was largely unreacted (Fig. 3*a* and *b*, lane d). Exposure of phospho-FixJ to 55 °C for 5 min under neutral conditions did not result in hydrolysis of the phosphate (data



a
FixL
1
86
220
464
b
FixL*
86
220
464
FixJ
1
120
204
c
P/O$_{lac}$,SD
fixL ⟶
fixJ ⟶
pGG820
thr met ile thr pro ser leu ala ala gly Arg86
atg acc atg att acg cca agc ttg gct gca ggt CGG

FIG. 1 Recombinant *fixLJ* region and its protein products. *a*, The structure of full-length FixL is schematically represented. ■, Hydrophobic stretches of amino acids. ▨, Regions of homology to other two-component systems. The numbers refer to amino-acid positions from David *et al.*[3]. *b*, Structures of FixL* and FixJ purified in this study. Positions of relevant amino acids are indicated. *c*, Recombinant genes that encode FixL* and FixJ. The DNA and amino-acid sequences of the *lacZ–fixL* junction are also shown. The genes are on a high-copy-number plasmid, pGG820, derived from pUC9. ▩, The *lac* promoter, operator and ribosome-binding site (*lac P/O*, RBS); □, sequences encoding FixL* and FixJ.

METHODS. Plasmid pGG820 was obtained in three steps. The initial plasmid for the constructions was pMW2 (M. Weinstein, unpublished data). Plasmid pMW2 consists of a 3.15-kilobase (kb) *Bam*HI fragment of *R. meliloti* DNA, containing the *fixLJ* operon, cloned into the *Bam*HI site of pUC9. First, plasmid pGG8, which contains only pUC9 sequences and the 5′ region of *fixL* was constructed. A 1.88-kb *Eco*RI fragment containing the 3′ region of the *fixLJ* operon was excised from pMW2, and pGG8 was produced by religating the vector fragment with T4 DNA ligase. In pGG82, the 86th codon of *fixL* is fused to the pUC9 polylinker region in-frame with first six *lacZ* codons. This was accomplished by excising a 0.63-kb *Acc*I–*Rsr*II region from pGG8, treating the vector fragment with Klenow and dNTPs to generate blunt DNA ends, then religating with T4 DNA ligase. Finally, the 3′ region of the *fixLJ* operon was reconstructed in pGG820 by cutting pGG82 with *Eco*RI and ligating it to the 1.88-kb *Eco*RI fragment from pMW2. The relevant regions of pGG820 are shown in Fig. 1*c*.

not shown). These results are consistent with the presence of an acyl phosphate linkage in phospho-FixJ and with the properties of the phosphorylated regulator proteins of other prokaryotic regulatory systems[10-12].

The visible spectrum of a solution of purified FixL* from 350 to 650 nm is that of a haemoprotein (Fig. 4). The absorption maxima of aerobic FixL*, at 576.5 (α), 542.5 (β) and 417 nm (γ, or Soret) and their relative heights are characteristic of haemoproteins. Oxyhaemoglobin, for example, has peaks of 576 (α), 541 (β) and 415 (γ) (Fig. 4*a*). Deoxygenation of the FixL*

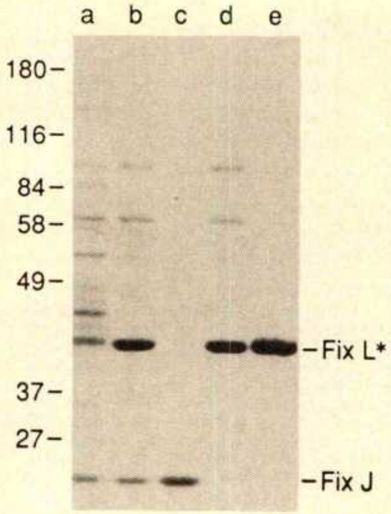


FIG. 2 Purification of FixL* and FixJ. Protein from each purification step was examined by electrophoresis on SDS–polyacrylamide gels (11%). Each lane contains 5 μg. The samples were: high-spin supernatant (lane a); fractions pooled after DEAE-Sephacel (lane b); FixJ fractions pooled after Sephacryl S-200 (lane c); FixL* fractions pooled after Sephacryl S-200 (lane d); FixL* fractions pooled after FPLC on a MonoQ column (lane e). M_r markers ($M_r \times 10^{-3}$; left) and the bands corresponding to FixL* and FixJ are indicated.
METHODS. The *E. coli* host strain was TG1 [*supE hsd* Δ*5 thi* Δ(*lac proAB*) *F*′(*traD36 proAB*$^+$ *lacI*^{q} *lacZ* Δ*M15*)][14]. This strain was transformed with the *fixLJ* plasmid pGG820 and the *nifA* promoter test plasmid pCHK57[1]. Strain TG1(pCHK57, pGG820) was maintained in M9 medium supplemented with 10 mM glucose, 1 μg ml^{-1} thiamine, 250 μg ml^{-1} penicillin G, and 15 μg ml^{-1} tetracycline. Before induction, a concentrated inoculum was prepared from a logarithmic culture grown in the above medium at 37 °C. LB, containing 250 μg ml^{-1} penicillin G and 15 μg ml^{-1} tetracycline, was inoculated to a starting 600 nm absorbance of 0.05. When the absorbance at 600 nm of the culture reached 0.4–0.5, IPTG was added to a final concentration of 1 mM, and the cells were collected after 5 h at 37 °C. A cell pellet obtained from 5 litres culture (20 g wet cells) was washed then resuspended in 80 ml buffer A (20 mM Tris–HCl, pH 7.8, 125 mM NaCl, 5% (v/v) glycerol, 10 mM β-mercaptoethanol, and 1 mM PMSF). The cells were lysed by 3-min sonication. Unbroken cells were removed by centrifugation at 3,000 r.p.m. (Sorvall rotor SS34) for 10 min. The crude extracts were then spun at 45,000 r.p.m. for 30 min (Ti60 rotor). The supernatant was directly applied to a DEAE-Sephacel column (2.5 × 12 cm) pre-equilibrated with buffer A, and the column was washed with 150 ml of the same buffer. Proteins were then eluted with 400 ml of a linear NaCl gradient from 125 to 400 mM, at a flow rate of 30 ml h^{-1}. The fractions containing FixL* and FixJ eluted at around 200 mM NaCl. The pooled fractions (50 ml) were concentrated to a volume of 1 ml by precipitation from 40% saturated ammonium sulphate and applied onto a Sephacryl S-200 column (2 × 125 cm) equilibrated with 20 mM Tris–HCl, pH 7.8, 150 mM NaCl, 5% glycerol and 10 mM β-mercaptoethanol. Elution was performed with the same buffer at a flow rate of 3 ml h^{-1}. The fractions having pure FixJ were combined and stored at −70 °C. Purified FixJ reacted positively with FixJ-specific antibody prepared by A. Lois. The fractions containing FixL* were further purified on a FPLC MonoQ HR5/5 1-ml column equilibrated with buffer B (20 mM Tris–HCl, pH 9.0, 125 mM NaCl, 5% glycerol and 10 mM β-mercaptoethanol). Protein was loaded onto the column in buffer B and eluted with a 25-ml linear 125–750 mM NaCl gradient at a flow rate of 30 ml h^{-1}. The fractions that eluted at 480 mM NaCl contained pure FixL*. Purification procedures were done at 4 °C. All chromatographic media were purchased from Pharmacia. Protein was measured by the Bradford method (Bio-Rad), using BSA as the standard. Initially, both FixL* and FixJ were detected by phosphorylation assays. Later, FixL* was monitored by absorption measurements near its absorption maximum (420 nm). The Sephacryl S-200 column was calibrated with a mixture of the M_r standards β-amylase (200K), alcohol dehydrogenase (150K), BSA (66K), carbonic anhydrase (29K) and cytochrome *c* (12.4K).

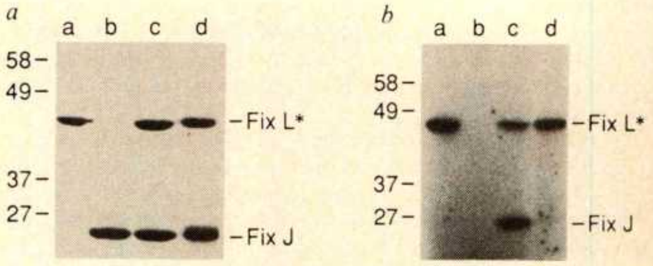


FIG. 3 Phosphorylation of FixL* and FixJ with [γ-^{32}P]ATP. Each purified protein present (1.9 μM FixL*, 3.6 μM FixJ) was incubated for 5 min in standard phosphorylation buffer in air. FixL* alone, lane a; FixJ alone, lane b; FixL* plus FixJ, lane c; FixL* plus FixJ treated 5 min at 55 °C in 0.5 M NaOH after phosphorylation, lane d. Products were analysed by Coomassie brilliant blue staining (*a*) or autoradiography (*b*) of SDS–polyacrylamide gels of the reaction mixtures after electrophoresis. The Coomassie-stained lanes had three times more sample. M_r markers ($M_r \times 10^{-3}$; left) and the positions of FixL* and FixJ are shown.
METHODS. Protein (2–7 μM) in 12.5 μl 60 mM Tris–HCl, pH8.0, 60 mM KCl, 5% (v/v) glycerol and 1.2 mM dithiothreitol was equilibrated for 5 min at 23 °C. Phosphorylation was initiated by adding 2.5 μl of a solution of 2.4 mM unlabelled ATP, 2.4 mM MgCl$_2$ and 10 μCi [γ-^{32}P]ATP (Amersham). After 5 min of incubation at room temperature, the reaction was stopped with 5 μl SDS buffer (125 mM Tris–HCl, pH 6.8, 8 mM EDTA, 4% (w/v) SDS, 8% (v/v) β-mercaptoethanol, 20% (v/v) glycerol and 0.02% (w/v) bromophenol blue). To study the stability of the phosphate linked to FixJ, the reaction products were treated with 5 μl 2 M NaOH, 8 mM EDTA for 5 min at 55 °C. The mixture was then neutralized with HCl, and SDS buffer was added. The samples were electrophoresed on an SDS–polyacrylamide gel (10–11%). The gel was dried in a vacuum, and the labelled proteins were detected by autoradiography.

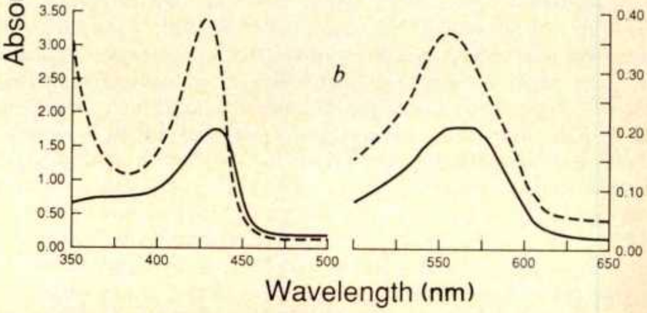


FIG. 4 Spectra of oxygenated and deoxygenated FixL*. Aerobic FixL* (13.4 μM monomer) in 20 mM Tris–HCl, pH 8.5, 150 mM NaCl, 5% (v/v) glycerol and 10 mM β-mercaptoethanol (*a*, solid curve); identical FixL* solution after it was made anaerobic (*b*, solid curve); oxyhaemoglobin (*a*, dashed curve); deoxyhaemoglobin (*b*, dashed curve). Wavelength scans were collected on a Uvicon 930 spectrophotometer (Kontron Instruments) in 1-cm path length quartz cells.

haem *in vacuo* resulted in absorption shifts very similar to those observed for deoxyhaemoglobin (Fig. 4*b*). The α and β bands are replaced by a single peak at 567.5 nm, and the Soret band is shifted to 433.5 nm. The corresponding deoxyhaemoglobin peaks were at 555 and 430 nm (Fig. 4*b*). These changes were completely reversible, demonstrating that FixL* retains its ability to respond to oxygen. The extinction coefficient of monomeric oxy-FixL* at 417 nm is roughly $1.6 \times 10^5\ \mathrm{M}^{-1}\ \mathrm{cm}^{-1}$, on the basis of protein assays and absorbance at 417 nm. Pyridine haemochrome assay suggests that FixL* contains one mole of haem per monomer.

FixL* is a novel haemoprotein (Fig. 4) with kinase activity (Fig. 3). We propose that FixL has at least three distinct regions (Fig. 1). The homologous region (residues 220-464) houses those functions that are common to the two-component sensors, such as ATP binding, partner recognition and phosphorylation (reviewed in refs 5-9). The hydrophobic region (residues 1-85) probably anchors FixL to the cell membrane. These residues, absent in FixL*, are unnecessary for haem binding or phosphorylation. They may stabilize the protein *in vivo* or protect the haem from oxidation. Membrane attachment may also enhance or modify the functions of FixL, or FixL may communicate with other membrane proteins. An extracytoplasmic loop could lie in the deleted hydrophobic region of FixL*. The removal of the extracytoplasmic loop from VirA, in the agrobacterial virulence system, did not eliminate the *in vivo* response to acetosyringone, indicating that the site of acetosyringone-sensing could be intramembranous or cytoplasmic[13]. The remaining region of FixL (residues 86-219) is likely to contain the haem attachment site, that is, the sensing portion of the molecule. This region is most probably on the cytoplasmic side of the membrane, because no hydrophobic stretches separate it from the enzymatic domain. Histidines are expected to have an important role in the function of this molecule, because they are commonly involved in haem coordination and are the sites of phosphorylation in several proteins homologous to FixL[10,15,16]. That FixL is a haemoprotein with kinase activity raises the question of whether the oxygenation state of the haem moiety affects its kinase activity. Experiments are in progress to answer this question and to provide evidence for a linkage between the phosphorylation events involving FixL and FixJ and signal transduction in nitrogen fixation. ☐

Received 12 November 1990; accepted 22 January 1991.

1. Ditta, G., Virts, E., Palomares, A. & Kim, C.-H. *J. Bact.* **169,** 3217-3223 (1987).
2. Virts, E. L., Stanfield, S. W., Helinski, D. R. & Ditta, G. S. *Proc. natn. Acad. Sci. USA* **85,** 3062-3065 (1988).
3. David, M. *et al. Cell* **54,** 671-683 (1988).
4. Nixon, B. T., Ronson, C. W. & Ausubel, F. M. *Proc. natn. Acad. Sci. USA* **83,** 7850-7854 (1986).
5. Stock, A. M. *et al. Cold Spring Harb. Symp. quant. Biol.* **53,** 49-57 (1989).
6. Stock, J. B., Ninfa, A. J. & Stock, A. M. *Microbiol. Rev.* **53,** 450-490 (1989).
7. Stock, J. B., Stock, A. M. & Mottonen, J. M. *Nature* **344,** 395-400 (1990).
8. Albright, L. M., Huala, E. & Ausubel, F. M. *A. Rev. Genet.* **23,** 311-336 (1989).
9. Gross, R., Arico, B. & Rappouli, R. *Molec. Microbiol.* **3,** 1661-1667 (1989).
10. Weiss, V. & Magasanik, B. *Proc. natn. Acad. Sci. USA* **85,** 8919-8923 (1988).
11. Forst, S., Delgado, J. & Inouye, M. *Proc. natn. Acad. Sci. USA* **86,** 6052-6056 (1989).
12. Bourret, R. B., Hess, J. F. & Simon, M. L. *Proc. natn. Acad. Sci. USA* **87,** 41-45 (1990).
13. Melchers, L. S. *et al. EMBO J.* **8,** 1919-1925 (1989).
14. Gibson, T. J. thesis, Cambridge Univ., UK (1984).
15. Hess, J. F., Bourret, R. B. & Simon, M. I. *Nature* **336,** 139-143 (1988).
16. Jin, S. *et al. J. Bact.* **172,** 525-530 (1990).

ACKNOWLEDGEMENTS. We thank P. Saltman and B. Dyke for discussions, comments on the manuscript and assistance with the spectroscopy; P. Geidushek for comments on the manuscript; and M. Adams and G. Anders for help with immunizations. This work was supported by the NSF. M.G.-G. is supported by a Fellowship from the NIH.

Single-site enzymatic cleavage of yeast genomic DNA mediated by triple helix formation

Scott A. Strobel & Peter B. Dervan*

Division of Chemistry and Chemical Engineering,
California Institute of Technology, Pasadena,
California 91125, USA

PHYSICAL mapping of chromosomes would be facilitated by methods of breaking large DNA into manageable fragments, or cutting uniquely at genetic markers of interest. Key issues in the design of sequence-specific DNA cleaving reagents are the specificity of binding, the generalizability of the recognition motif, and the cleavage yield. Oligonucleotide-directed triple helix formation is a generalizable motif for specific binding to sequences longer than 12 base pairs within DNA of high complexity[1-3]. Studies with plasmid DNA show that triple helix formation can limit the operational specificity of restriction enzymes to endonuclease recognition sequences that overlap oligonucleotide-binding sites[4,5]. Triple helix formation, followed by methylase protection, triple helix-disruption, and restriction endonuclease digestion produces near quantitative cleavage at the single overlapping triple helix–endonuclease site[4,5]. As a demonstration that this technique may be applicable to the orchestrated cleavage of large genomic DNA, we report the near quantitative single-site enzymatic cleavage of the *Saccharomyces cerevisiae* genome mediated by triple helix formation. The 340-kilobase yeast chromosome III was cut uniquely at an overlapping homopurine–*Eco*RI target site 27 base pairs long to produce two expected cleavage products of 110 and 230 kilobases. No cleavage of any other chromosome was detected. The potential generalizability of this technique, which is capable of near quantitative cleavage at a single site in at least 14 megabase pairs of DNA, could enable selected regions of chromosomal DNA to be isolated without extensive screening of genomic libraries.

Chemical reagents and biological methods that infrequently cleave double helical DNA are being developed for genomic mapping[1-12]. Recently, Szybalski and coworkers reported an 'Achilles heel cleavage' technique that limits restriction enzyme cleavage to an overlapping *lac* repressor-endonuclease site[11,12]. Protein-mediated Achilles heel cleavage may, however, not be readily generalized to unique cleavage at selected genetic markers owing to the paucity of applicable DNA-binding proteins relative to the number of sites in megabase genomic DNA. To achieve orchestrated cleavage at selected genetic markers in human DNA by this approach may require the protein-binding sequence to be artificially inserted into the chromosome at precise locations. A more general method for recognition of endogenous DNA sequences might be a chemical approach based on oligonucleotide-directed triple helix formation[1]. Pyrimidine oligonucleotides bind specifically to purine sequences in duplex DNA to form a local triple-helix structure[1-5,13,14]. The generalizable code for triple-helix specificity is derived from thymine (T) binding to adenine–thymine base pairs (T·AT base triplet)[15] and N3 protonated cytosine (C+) binding to guanine–cytosine base pairs (C+GC base triplet)[16,17]. Higher affinity oligonucleotides can be obtained by substituting 5-bromouracil for thymine and 5-methylcytosine for cytosine[18]. Moreover, the number of potential target sequences amenable to recognition by the triple helix motif can be extended to some mixed purine–pyrimidine sequences[19,20]. By replacing the *lac* repressor protein in the Achilles heel technique with triple-helix-mediated protection, one combines the strengths of the two approaches; a general chemical method for recognition of DNA sequences in the range of 15-20 base pairs (bp) with the cleavage efficiency of restriction enzymes (Fig. 1).

To demonstrate single-site enzymatic cleavage of the yeast genome by this technique, a sequence containing an overlapping 24-bp purine tract and 6-bp *Eco*RI site was inserted proximal to the *LEU2* (ref. 21) gene on the short arm of chromosome

---

* To whom correspondence should be addressed.

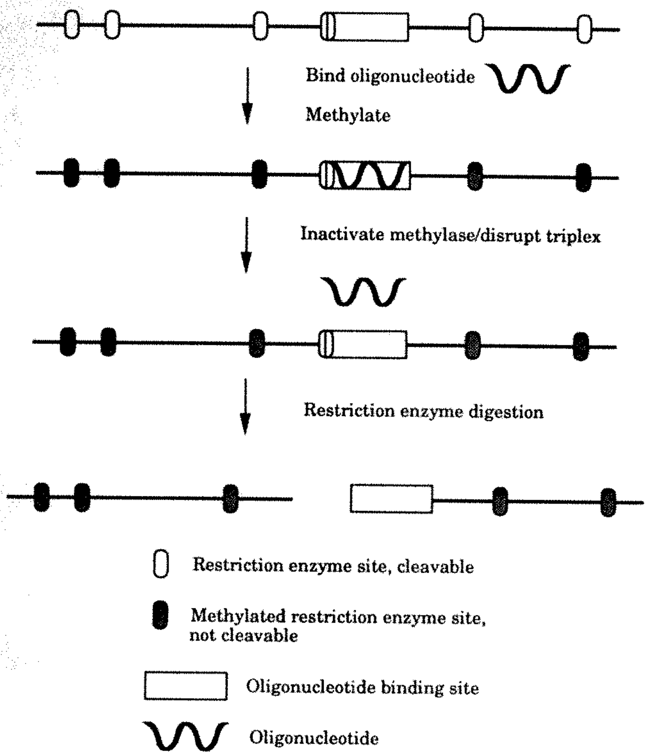

Bind oligonucleotide
Methylate
Inactivate methylase/disrupt triplex
Restriction enzyme digestion
Restriction enzyme site, cleavable
Methylated restriction enzyme site, not cleavable
Oligonucleotide binding site
Oligonucleotide

FIG. 1 General scheme for single-site enzymatic cleavage of genomic DNA by oligonucleotide-directed triple helix formation. Chromosomal DNA is equilibrated with an oligonucleotide in a methylase compatible buffer containing polycation. *Eco*RI methylase which methylates the central adenines of the sequence 5′-GAATTC-3′ and renders the sequence resistant to cleavage by *Eco*RI restriction endonuclease, is added and allowed to proceed to completion. The methylase is inactivated and the triple helix is disrupted at 55 °C in a high-pH buffer containing detergent. After washing extensively, the chromosomal DNA is re-equilibrated in restriction enzyme buffer and cut to completion with *Eco*RI restriction endonuclease. The cleavage products are separated by pulsed-field gel electrophoresis and efficiencies quantitated by Southern blotting.

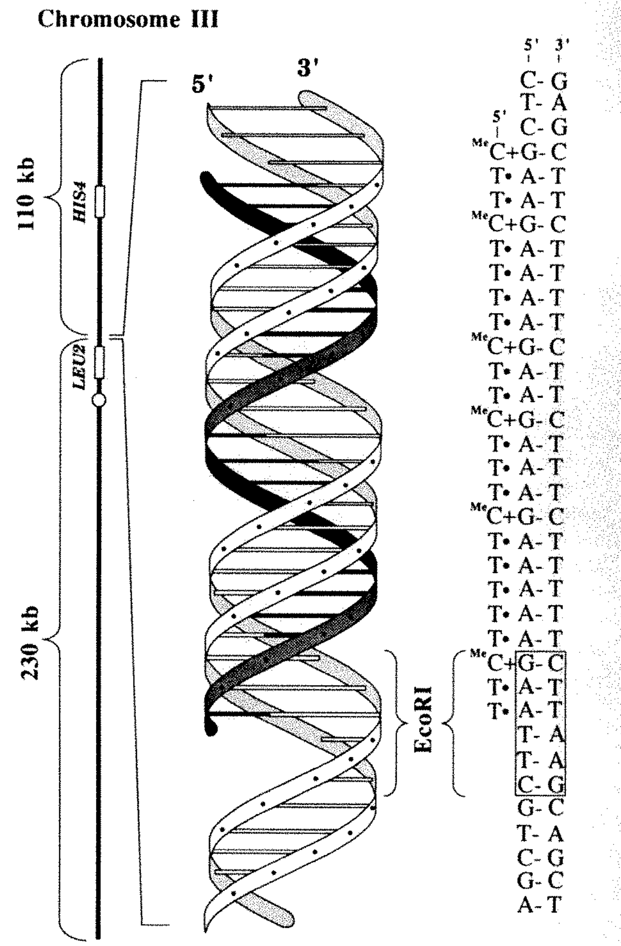


FIG. 2 Left, genetic map of *S. cerevisiae* chromosome III. The locations of the *HIS4* and *LEU2* loci (boxes), the centromere (circle), and the triple helix–*Eco*RI target site are indicated. The expected sizes of the cleavage products are shown. Right, schematic diagram of the triple helix complex overlapping the *Eco*RI restriction–methylation site. The pyrimidine oligonucleotide is bound in the major groove parallel to the purine strand of the DNA duplex, and covers half the *Eco*RI site.

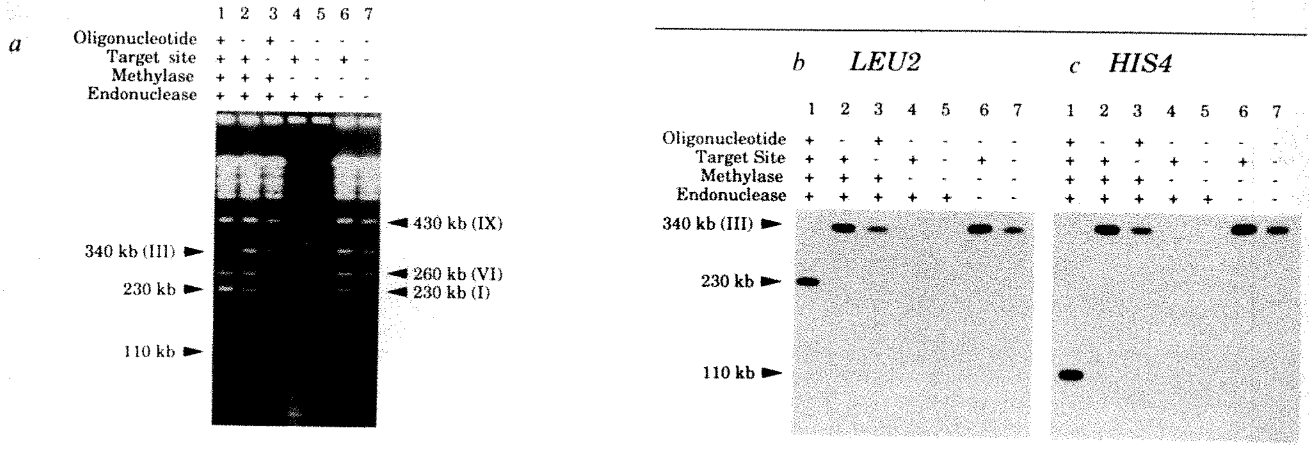


FIG. 3 Single-site enzymatic cleavage of yeast genomic DNA with reagents indicated above figure. 1, Yeast chromosomal DNA embedded in low-melting-point agarose (~50 μl) was washed twice in 1.0 ml of 100 mM NaCl, 100 mM Tris-HCl, 10 mM EDTA, 2 mM spermine, pH 7.6 for 10 min, decanted, and overlayed with 150 μl of the same buffer. Oligonucleotide was added to 1 μM final concentration and incubated 8 h at 22 °C. 2, Bovine serum albumin (100 μg ml^{-1}), *S*-adenosylmethionine (160 μM) and *Eco*RI methylase (80 units) were added to the overlay and incubated with shaking for 3 h. 3, The triple helix was destabilized and the methylase was simultaneously inactivated at 55 °C in 1.0 ml of 1% lauryl sarcosyl, 100 mM Tris-HCl pH 9.5, 10 mM EDTA for 30 min. The oligonucleotide and detergent were then removed with 4 × 10 min washes (1.0 ml) with 10 mM Tris-HCl pH 9.5, 10 mM EDTA. 4, The agarose plug was washed twice with 1.0 ml 2 × potassium glutamate restriction enzyme buffer (200 mM potassium glutamate, 50 mM Tris-acetate, pH 7.6, 20 mM magnesium acetate, 100 μg ml^{-1} BSA, 1 mM 2-mercaptoethanol), decanted, overlayed with 150 μl of buffer and the DNA digested to completion with 20 U *Eco*RI restriction endonuclease for 6 h at 22 °C. After digestion the enzyme was heat-inactivated at 55 °C for 10 min and the DNA loaded onto a 1% agarose, 0.5 × TBE pulsed-field gel at 14 °C, 200 V. Switch times were ramped from 10–40 s for 18 h followed by ramping from 60–90 s for 6 h. Products were visualized by ethidium bromide staining (*a*) and Southern blotting with *LEU2* (*b*) and *HIS4* (*c*) chromosome markers. Cleavage efficiencies were measured using storage phospho imaging plates with a Molecular Dynamics 400S PhosphoImager.

III (Fig. 2) (ref. 3). Oligonucleotides designed to form triple helix complexes that overlap half of the *Eco*RI recognition site were synthesized with CT, ^{Me}CT or ^{Me}C^{Br}U nucleotides[18]. The genetic map of yeast chromosome III (refs 22, 23) and affinity cleaving data[3] indicate that cleavage at the target site should produce two fragments 110 ± 10 and 230 ± 10 kilobases (kb) in size (Fig. 2).

Resolution of total yeast chromosomal DNA by pulsed-field gel electrophoresis[24,25] revealed that chromosome III was cut exclusively at the target site when a ^{Me}CT oligonucleotide was used for triple helix formation at pH 7.6 (Fig. 3, lane 1). No cleavage was detected on any other chromosomes under these conditions nor was cleavage observed in the absence of oligonucleotide (lane 2) or in a yeast strain lacking the target sequence (lane 3). The expected 110-kb product was visualized with ethidium bromide staining and confirmed by Southern blotting with a *HIS4* (ref. 26) marker (Fig. 3*c*). The 230-kb product comigrated with chromosome I, but was detected by Southern blotting with a *LEU2* (ref. 24) marker (Fig. 3*b*). The cleavage efficiency was 94 ± 2%. Similar efficiencies were seen with a CT oligonucleotide up to pH 7.4 and ^{Me}CT and ^{Me}C^{Br}U oligonucleotides past pH 7.8 (Fig. 4). The cleavage efficiency with all oligonucleotides was gradually reduced with longer methylation times, suggesting that the oligonucleotide dissociation rate might be the limiting factor for efficiency in this system[27].

The specificity of triple helix formation has been shown to be pH-dependent[1-3]. By lowering the pH, sequences of near but imperfect similarity can be bound and cleaved[1-3]. In agreement with this observation, secondary cleavage sites were revealed as a function of oligonucleotide composition and pH (Fig. 4). Cleavage at the secondary site was preferentially reduced by longer methylation times, suggesting differential oligonucleotide dissociation rates between the primary and secondary target sites. Cleavage at all secondary sites could be eliminated at a threshold pH for each oligonucleotide.

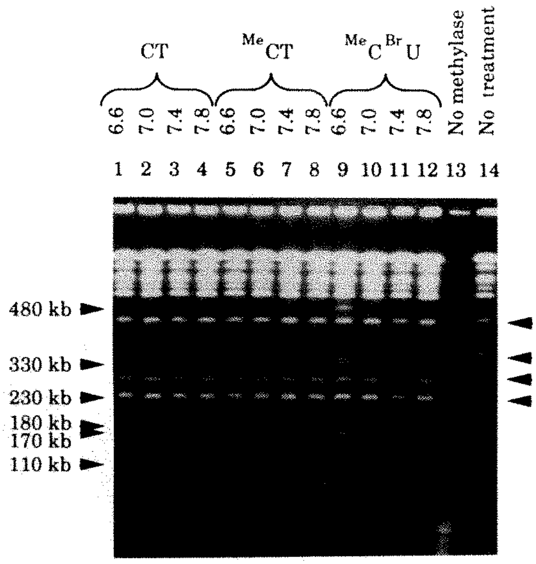


FIG. 4 Triple-helix-mediated enzymatic cleavage of the yeast genome as a function of oligonucleotide composition and pH. Lanes 1–12, reactions on a yeast strain containing the triple helix target site with oligonucleotides (CT, ^{Me}CT and ^{Me}C^{Br}U) and pH values (6.6, 7.0, 7.4 and 7.8) as indicated above figure. Methylation time was 4.5 h. 170-kb and 650-kb (unresolved) secondary cleavage products were observed with both ^{Me}C substituted oligonucleotides at or below pH 7.4. The cleavage site can be assigned to chromosome II (820 kb) by two-dimensional pulsed-field gel electrophoresis in which the DNA was triple helix-protected and methylated before the first dimension and restriction enzyme-digested before the second dimension (data not shown). Additional secondary cleavage sites were observed with the ^{Me}C^{Br}U oligonucleotide at pH 6.6 (lane 9). The 180- and 480-kb products were assigned to chromosome XI (660 kb), and the 330 and 780 kb (unresolved) products were assigned to the VII/XV doublet (1,100 kb) by the same method (data not shown).

Whereas affinity cleaving using oligonucleotide-EDTA·Fe identifies all sites of oligonucleotide binding[1-3], triple-helix-mediated endonuclease cleavage exposes only those sequences that also partially overlap a restriction site[4,5]. The sequence requirement of a methylation-restriction site increases the cleavage specificity but reduces the number of available sites. To partially overcome this limitation, other commercially available methylation and restriction enzyme pairs with homopurine half sites were tested on plasmid DNA. In addition to *Taq*I (ref. 4) and *Eco*RI (ref. 5), single-site protection of plasmid DNA was possible with *Msp*I, *Hpa*II and *Alu*I methylases. *Bam*HI, *Hae*III and dam methylases could potentially be used, but remain untested.

The generalizability of triple helix-mediated enzymatic cleavage affords high specificity that can, in principle, be customized to unique genetic markers without artificial insertion of a target sequence. The use of degenerate oligonucleotides in this technique to rapidly screen genetic markers for overlapping triple-helix methylation-restriction sites could make it possible to cut chromosomal DNA uniquely and efficiently at endogenous sites with minimal sequence information (S.A.S. and P.B.D., unpublished observations). ☐

Received 5 October; accepted 20 December 1990.

1. Moser, H. E. & Dervan, P. B. *Science* **238**, 645–650 (1987).
2. Strobel, S. A., Moser, H. E. & Dervan, P. B. *J. Am. chem. Soc.* **110**, 7927–7929 (1988).
3. Strobel, S. A. & Dervan, P. B. *Science* **249**, 73–75 (1990).
4. Maher, L. J., Wold, B. & Dervan, P. B. *Science* **245**, 725–730 (1989).
5. Hanvey, J. C., Shimizu, M. & Wells, R. D. *Nucleic Acids Res.* **18**, 157–161 (1990).
6. Barlow, D. P. & Lehrach, H. *Trends Genet.* **3**, 167–171 (1987).
7. Wenzlau, J. M., Saldanha, R. J., Butow, R. A. & Perlman, P. S. *Cell* **56**, 421–430 (1989).
8. Delahodde, A. *et al. Cell* **56**, 431–441 (1989).
9. Patel, Y., Van Cott, E., Wilson, G. G. & McClelland, M. *Nucleic Acids Res.* **18**, 1603–1607 (1990).
10. Weil, M. D. & McClelland, M. *Proc. natn Acad. Sci. U.S.A.* **86**, 51–55 (1989).
11. Koob, M., Grimes, E. & Szybalski, W. *Science* **241**, 1084–1086 (1988).
12. Koob, M. & Szybalski, W. *Science* **250**, 271–273 (1990).
13. Praseuth, D. *et al. Proc. natn Acad. Sci. U.S.A.* **85**, 1349–1353 (1988).
14. Lyamichev, V. I. *et al. Nucleic Acids Res.* **16**, 2165–2178 (1988).
15. Felsenfeld, G., Davies, D. R. & Rich, A. *J. Am. chem. Soc.* **79**, 2023–2024 (1957).
16. Lipsett, M. N. *J. biol. Chem.* **239**, 1256–1260 (1964).
17. Rajagopal, P. & Feigon, J. *Nature* **339**, 637–640 (1989).
18. Povsic, T. J. & Dervan, P. B. *J. Am. chem. Soc.* **111**, 3059–3061 (1989).
19. Griffin, L. C. & Dervan, P. B. *Science* **245**, 967–971 (1989).
20. Horne, D. A. & Dervan, P. B. *J. Am. chem. Soc.* **112**, 2435–2437 (1990).
21. Andreadis, A., Hsu, Y. P., Kohlhaw, G. B. & Schimmel, P. *Cell* **31**, 319–325 (1982).
22. Mortimer, R. K. & Schild, D. *Microbiol. Rev.* **49**, 181–212 (1985).
23. Carle, G. F. & Olson, M. V. *Proc. natn Acad. Sci. U.S.A.* **82**, 3756–3760 (1985).
24. Schwartz, D. C. & Cantor, C. R. *Cell* **37**, 67–75 (1984).
25. Carle, G. F. & Olson, M. V. *Nucleic Acids Res.* **12**, 5647–5664 (1984).
26. Keesey, J. K., Bigelis, R. & Fink, G. R. *J. biol. Chem.* **254**, 7427–7433 (1979).
27. Maher, L. J., Dervan, P. B. & Wold, B. *Biochemistry* **29**, 8820–8826 (1990).

ACKNOWLEDGEMENTS. We thank J. Hanish for helpful discussions. The work was supported by the National Institutes of Health and the Howard Hughes Medical Institute (predoctoral fellowship to S.A.S.).

ERRATUM

Meteoritic silicon carbide: pristine material from carbon stars

Roy S. Lewis, Sachiko Amari & Edward Anders

Nature **348**, 293–302 (1990)

IN this article, the second affiliation for Edward Anders was omitted. In addition to the Chicago address, he is attached to the Physikalisches Institut der Universität, CH-3012 Bern, Switzerland.

The first step-by-step guide to human gene mapping

Human Gene Mapping Techniques

By **Helen Donis-Keller, PhD,** Washington University School of Medicine, St. Louis, Missouri

Often a first step towards the understanding of the molecular basis of an inherited disorder is pinpointing the disease causing gene to a chromosome region. Once the chromosome region is identified, experiments to directly clone and characterize the gene can then be undertaken.

Human Gene Mapping Techniques is a step-by-step guide to designing a mapping project, identifying the chromosome location of a gene, developing a high resolution genetic map, and cloning a gene once its chromosome location is known. Detailed laboratory procedures include establishment of permanent cell lines, genetic linkage analysis techniques, physical localization of DNA sequences by *in situ* hybridization, and chromosome walking using yeast artificial chromosome vectors (YACs). This is a practical guide that includes automated laboratory procedures, sources for equipment and reagents, and an extensive list of citations for additional reading. With a major emphasis on genetic linkage mapping, this book presents strategies for mapping genes for researchers who have little acquaintance with genetic mapping but some experience with molecular biology and gene cloning techniques.

Tentative Contents: Meiotic Genetic Linkage Mapping Methods • Radiation Hybrid Genetic Linkage Mapping • Linkage Mapping Using Male Meioses • *In Situ* Hybridization of Cloned DNA to Chromosomes • Somatic Cell Rodent-Human Hybrid Panel Analysis • Construction of Single Chromosome Deletion Panels • Chromosome Sorted Blots for Gene Localization • Data Acquisition, Storage, Access, Communications • Physical Mapping By Pulsed-Field Gels • Yeast Artificial Chromosomes (YAC) Cloning • Constructing Overlapping Clone Maps

December 1990, 250 pp., 0-333-53541-3, £27.95

Send orders to Globe Book Service Ltd., FREEPOST, Stockton House, 1 Melbourne Place, London WC2B 4LF, UK. Or call : 01-379-4687. We accept Access/MasterCard, Visa, Diners' Club and American Express. Please add £2.00 postage and packing. FREEPOST applies to UK only. (Globe book Services is a division of Macmillan, Publishers, Ltd.)

M

The world's most intensively cited
leukemia journal, now published by Macmillan

LEUKEMIA

EDITORS

C. Nicole Muller-Bérat, Denmark
William M. Crist, USA Edward S. Henderson, USA
Mel F. Greaves, UK

CLINICAL STUDIES
■
CELL BIOLOGY
■
MOLECULAR HEMATOLOGY

- Differentiation
- *In Vitro* Models
- Molecular Pharmacology
- Membrane Markers
- Oncogenes
- Gene Therapy
- Enzymatic Markers
- Stem Cells
- Virology
- Biological Modifiers
- Bone Marrow Transplantation
- Therapeutic Trials
- Cell Cycle
- Growth Factors
- Chromosomes

FROM RECENT ISSUES OF LEUKEMIA

OPEN FORUM:

Remarkably Long Survival of a Patient with Ph^1-Positive Chronic Myeloid Leukemia and 5′ bcr Rearrangement
B. Johansson, F. Mertens, T. Fioretos, S. Heim, U. Kristoffersson, N. Mandahl, C.R. Bartram & F. Mitelman

Cure of Acute Myelocytic Leukemia in Adults: A Reality *R.B. Geller, R. Saral, J.E. Karp, G.W. Santos & P.J. Burke*

CONGRESS LETTER:

Chronic Myelogenous Leukemia — The Unfolding Saga
J.M. Goldman, G. Grosveld, D. Baltimore & R.P. Gale

COMMENTARY:

The Sellafield Childhood Leukemia Cluster: Are Germline Mutations Responsible?
M.F. Greaves

ORIGINAL ARTICLES:

Induction of Genes for Transcription Factors by normal Hematopoietic Regulatory Proteins in the differentiation of Myeloid Leukemic Cells
Y. Shabo, J. Lotem & L. Sachs

Granulocyte Colony-Stimulating Factor, not Granulocyte-Macrophage Colony-Stimulating Factor, cooperated with Retinoic Acid on the Induction of Functional N-Formylmethionyl-Phenylalanine Receptors on HL-60 Cells
A. Sakashita, T. Nakamaki, N. Tsuruoka, Y. Honma & M. Hozumi

Serum Interleukin-2, Soluble IL-2 Receptors and Tumor Necrosis Factor-Alfa levels are significantly increased in Acute Myeloid Leukemia Patients
G. Cimino, S. Amadori, M.C. Cava, V. De Sanctis, M.C. Petti, O. Di Gregorio, C. Sgadari, L. Vegna, G. Cimino & F. Mandelli

Effects of Recombinant Human Granulocyte Colony Stimulating Factor and Granulocyte-Monocyte Colony Stimulating Factor on in Vitro Hemopoiesis in the Myelodysplastic Syndromes
A. Nagler, N. Ginzton, R. Negrin, D. Bang, T. Donlon & P. Greenberg

Is the mdr 1 Gene Relevant in Chronic Lymphocytic Leukemia?
J.A. Holmes, A. Jacobs, G. Carter, J.A. Whittaker, D.P. Bentley & R.A. Padua

Published on behalf of the Sven-Aage Killmann Foundation

Reader Service No.16

Subscription Rates

Monthly
ISSN 0887 6924

ONE YEAR	
Institutional	£202
Individual	£101
TWO YEARS	
Institutional	£404
Individual	£202

The individual rate is only available if you are paying with your **personal** cheque or credit card.

SEND FOR YOUR FREE SAMPLE COPY

To Dionne Stocking, Macmillan Press Ltd, Houndmills, Basingstoke
RG21 2XS, UK.

☐ Please send me a **FREE** sample copy of Leukemia

Name ____________________
Position ____________________
Address ____________________
____
____
Postal/Zip Code __________ Country __________

NAT

M

Nature Classified

LONDON Julie Skeet, 4 Little Essex St, London WC2R 3LF. Telephone: 071 872 0102. Fax: 071 240 2408.
NEW YORK – Marianne Ettisch, 65 Bleecker Street, New York, NY 10012. Telephone: (212) 477 9625. Fax: (212) 505 1364.
PARIS Clare Newell, BP No 804, 75828 Paris, Cedex 17. Tel: (1) 48 87 24 43. Fax: (1) 48 87 46 71.
MUNICH – Sasha Panter, Sandstrasse 41 8000 München 2. Telephone: (089) 52 40 82. Fax: (089) 5 23 22 22.
TOKYO – Phillip Hamill, Shin-Mitsuke Building (4F), 3-6 Ichigaya Tamachi, Shinjuku-ku, Tokyo 162. Telephone: (03) 3267 8751. Fax: (03) 3267 8746.

RATES UK & Rest of world – Display £24 per centimetre. Semi-Display £20 per centimetre. Minimum 3 cm. £3 is charged for a box number.
CONDITIONS The Publisher reserves the right to refuse, amend, withdraw or otherwise deal with all advertisements submitted to him at his absolute discretion and without explanation. The Publishers will not be liable for any loss occasioned by the failure of any advertisement to appear from any cause whatever, nor do they accept liability for printers' errors, although every care is taken to avoid mistakes.

CANCELLATIONS MUST BE RECEIVED NO LATER THAN 5PM ON THURSDAYS PRIOR TO ISSUE DATE.

DEPARTMENT OF BIOCHEMISTRY UNIVERSITY OF OXFORD

Oxford Glycobiology Institute

RESEARCH ASSISTANT IN NMR BR/355

The Oxford Glycobiology Institute is an interdisciplinary research centre, specialising in the structure and function of glycoconjugates which are of biochemical, pharmaceutical and medical interest. An important part of this programmes is to develop techniques suitable for glycoconjugate research. The NMR facility consists of a dedicated 500 MHz spectrometer with access to 360, 500 MHz machines. the Glycobiology Institute also have a Micro Vax 11, an FPS mini supercomputer and an Evans & Sutherland PS390. A position exists in the Institute for a Research Assistant initially for a five year period with the possibility of renewal. The successful candidate will have completed a PH.D. in any branch of science and will be part of a team interested in studying the structure and function of glycoconjugates by applying 2D NMR techniques and using molecular dynamics calculations. There will be the option of participating in the teaching programme within the Department of Biochemistry. Salary is on the RA1A Scale £11,399 - £18,165 per annum, according to age and experience, but more senior appointments may be made in appropriate circumstances.

Applications, including a curriculum vitae, and the names and addresses of three referees should be sent to:

The Senior Administrator, Dept. of Biochemistry,
South Parks Road, Oxford OX1 1QU.

AN EQUAL OPPORTUNITY EMPLOYER. (5802)A

DEPT OF BIOCHEMISTRY, SOUTH PARKS ROAD, OXFORD OX1 1QU

THE NATIONAL INSTITUTE OF IMMUNOLOGY, NEW DELHI

is looking for a

DIRECTOR OF THE INSTITUTE

from Indian citizens including those staying abroad and Non Resident Indians (NRIs) who should be an outstanding Scientist in the area of modern biology and should have excellent leadership and managerial qualities.

The mandate of the Institute is to carry out high quality basic and applied research in an end-to-end manner with the ultimate objective of developing new products of relevance to the country's pressing health problems.

The initial appointment will be made with a contract for 5 years and is renewable.

The Institute is also inviting Professorial level appointments in the areas of reproductive immunology, molecular, cellular and neuroimmunology, biophysics, structural and molecular imaging, and vaccinology and vaccine delivery systems.

Those who are interested may kindly contact as early as possible preferably by 1st week of April, 91, Prof V Ramalingaswami, Chairman, Search-cum-Selection Committee, National Institute of Immunology, New Delhi-110067, India (Fax 91-11-6862125; Tel: 2216155).

(W8304)A

MEDICAL EPIDEMIOLOGIST

The March of Dimes/California Birth Defects Monitoring Program (MOD/CBDMP) has an opening for a Medical Epidemiologist, located in the San Francisco Bay Area.

The Medical Epidemiologist is primarily responsible for independently conducting epidemiologic studies of congenital anomalies. Also, acts as principal investigator in developing and directing epidemiological studies about congenital anomalies and works within a group comprised of other epidemiologists, providing consultation and serving as a resource in the areas of reproductive biology and medicine.

Candidates should have an M.D. degree, be licensed to practice medicine in California, have completed residency in pediatrics, internal medicine & family practice, and be board eligible. Training in epidemiology and experience in conducting epidemiological research studies are required. Genetics training is preferred.

MOD/CBDMP offers a highly professional atmosphere with ample program resources to conduct epidemiological research. We offer a competitive salary plus an outstanding choice of fringe benefits and generous personal leave policy. Salary may also be augmented by up to 20% if the applicant can secure grant support.

Qualified individuals may submit curriculum vitae and three professional references by May 1, 1991 to:

John A. Harris, M.D., M.P.H., Chief
MOD/CBDMP
5900 Hollis Street, Suite A
Emeryville, CA 94608

March of Dimes
Preventing Birth Defects

Equal Opportunity Employer (NW6503)A

UNIVERSITY OF GLASGOW

A Post Doctoral Research Assistant

is required to undertake studies into biochemistry of root caries in teeth. The project will involve in vitro investigation of the mechanisms involved in the demineralisation which accompanies this disease.

Applicants should have expertise in general biochemical techniques. Some experience of protein chemistry would be an advantage.

The appointment will be for three years, on the Research Assistant scale, according to age and experience. The work will be carried out in the Departments of Pathological Biochemistry, Glasgow Royal Infirmary, and Oral Medicine and Pathology, Glasgow Dental Hospital. The two departments have well equipped laboratories, with extensive back up facilities.

Further details may be obtained from Dr Frances Dryburgh, Consultant Biochemist, Institute of Biochemistry, Royal Infirmary, Glasgow, G4 0SF with whom application, c.v. together with the names and addresses of two referees, should be lodged on or before 15th April, 1991. (5811)A

NIDA

NATIONAL INSTITUTE ON DRUG ABUSE

Post-Doctoral Position in Drug Abuse Research

The NIDA Addiction Research Center is the intramural research program of the National Institute on Drug Abuse. IRTA and Visiting Fellowship positions are presently available in the Preclinical Pharmacology Branch. Applications are invited for postdoctoral fellow appointments within the Behavioral Pharmacology and Genetics Laboratory. Successful candidates will join a group of researchers applying techniques from the fields of pharmacogenetics, behavioral genetics and neurochemistry to questions related to the mechanisms of action of abused substances. Positions are available to study neurochemical changes associated with chronic drug administration and genetic differences in response to drugs of abuse. Experience in these areas is desirable, but not essential. Duties include conducting experiments and writing manuscripts and reports. Positions are also available in the laboratory to study drug self-administration and physical dependence in rhesus monkeys, the cardiovascular effects of abused drugs, and behavioral and neurochemical assessments of transgenic mice. Candidates must have a Ph. D. degree and no more than 3 years relevant postdoctoral experience in either of these areas. The position is open to either foreign or U.S. citizens and has a current starting salary between $25,000 and $29,000 depending on the number of years of postdoctoral experience. The NIDA Addiction Research Center is an equal opportunity employer and specifically invites and encourages application from women, minorities, and the handicapped.

If interested, please send CV and statement of interest to:

Steven R. Goldberg, Ph. D. Chief
Behavioral Pharmacology and Genetics
Laboratory
NIDA Addiction Research Center
P.O. B 5180
Baltimore, MD 21224

(NW6516)A

The Alcohol, Drug Abuse and Mental Health Administration is an Equal Opportunity Employer. ADAMHA is smokefree.

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE

(University of London)

Department of Neurological Science

POST-DOCTORAL BIOCHEMIST

MUSCULAR DYSTROPHY (Mitochondrial Myopathies)

required to work on an exciting 3 year project, funded by Muscular Dystrophy Association (UK), designed to study the transport of proteins into mitchondria and the effect mitochondrial abnormalities have on this transport. Work will involve tissue culture, mitochondrial DNA analysis and the study of respiratory chain enzyme function.

For informal discussion telephone Dr A Schapira — 071-794 0500, ext. 4363.

Salary scale Research IA plus £1,767 London Allowance.

Applications (3 copies of curriculum vitae and names and addresses of two referees) should be sent to the School Office, RFHSM, Rowland Hill Street, London NW3 2PF (Tel: 071-794 0500, ext. 4974) from which full job description is available. Closing date 2 April 1991. Please quote reference NEUR/PDB/175.

(5809)A

ATMOSPHERIC SCIENTIST/MODELER

The Universities Space Research Association (USRA) is currently seeking a PhD-level scientist to work in a GLA-GCM Model Development Group within NASA/Goddard Space Flight Center's (Atmospheric) Global Modeling and Simulation Branch. The work will be oriented toward developing computer models for the planned Earth Observing System, with a focus on 4-D data assimilation and climate studies.

The position offers an exciting opportunity for a creative scientist. Goddard has become a world leader in applying space data and advanced computational technology to the task of better understanding the dynamics of and interaction between the atmosphere, the ocean and the land surface. Applicants must have a PhD in meterology or a related physical science discipline. Experience is highly desirable in one or more of the following areas: 4-D data assimilation, initialization, parameterization of physical processes, numerical analysis, objective analysis, CRAY Fortran programming, and/or computational hydrodynamics. Salary will be competitive, commensurate with experience. A relocation allowance is available if required.

The Universities Space Research Association is a non-profit university consortium, chartered to foster cooperation between universities and the US Government toward the advancement of research and education in space science and technology. USRA is an equal opportunity employer. Applications received before April 30, 1991, will receive full consideration.

Send curriculum vitae and contacts for reference to: Dr Frank J Kerr, Position 911, USRA Visiting Scientist Program, Mail Code 610.3, NASA/Goddard SFC, Greenbelt, MD 20771. Telephone: (301) 286-5057.

(NW6506)A

Johannes Gutenberg-Universität Mainz

Naturwissenschaftlich-Medizinisches Forschungszentrum

Biochemist/Molecular Biologist

Postdoctoral position in an active research group with excellent, long term funding open. Members of the team have a strong background in molecular biology and immunology. The research involves the regulation and cellular expression of membrane proteins in human tumors. The candidate should have experience in protein biochemistry including HPLC purification methods.

A fully equipped laboratory can be provided.

The salary is according to the German BAT IIa. The appointment will be available for about two years initially.

Handicapped with comparable qualifications will be considered favourably.

Applications should be sent to: Prof Dr W Dippold, I. Medical Clinic, Langenbeckstr 1, 6500 Mainz, Germany.

(W8294)A

SFI is a Vienna-based pharmaceutical center and represents an important element in the international drug-discovery effort of the SANDOZ group.

The main focus of our activities is preclinical research in:

IMMUNOPHARMACOLOGY DERMATOLOGY and ANTI-RETROVIRAL THERAPY

We are seeking a

PHARMACOLOGIST

(with experience in immunobiology and/or microbiology)

on the B.V.M. or M.D. level to join our multidisciplinary team. The group is undertaking major research programs to explore recombinant protein technology for therapeutic agents. From an established position in the field of hematopietic growth factors, we are diversifying into research in immunological disorders.

We are especially interested in attracting applications who have experience in vivo work and wish to utilize these skills in the evaluation of new therapeutic agents.

The research will include in vivo work at our primate colony.

At present SANDOZ Research Institute has a permanent scientific staff of 150 members which is supplemented by graduate students, post-doctoral fellows and visiting scientists. A number of researchers also have teaching appointments at local universities or lecture abroad. The scientific atmosphere at SANDOZ Research Institute is increasingly international, and English is the in-house working language. The scientific environment is further enriched by the presence of university groups occupying laboratories at the SANDOZ Research Institute. Scientific, administrative and technical support is provided by a staff of 90. Research facilities at the Institute are state-or-the-art and include biohazard containment units and extensive housing for both large and small animals.

Vienna is not only a cosmopolitan city offering a fascinating range of cultural events, but scientifically, it is attracting an increasing number of international enterprises involved in molecular genetics and biotechnology. The opportunities for a fruitful, intellectually stimulating dialogue are further enhanced by close cooperation with other SANDOZ research units and with several leading scientific centers.

If you find our offer challenging and have the necessary qualifications, please submit a written application, including C.V. to

Dr Harwig Kainz, Personnel Manager,
SANDOZ Forschungsinstitut, Brunnerstr. 59
A-1235 Vienna, Austria

(W8296)A

SANDOZ
Forschungsinstitut Ges.m.b.H.

MULLARD SPACE SCIENCE LABORATORY
UNIVERSITY COLLEGE LONDON

UCL

Holmbury St Mary
Dorking, Surrey RH5 6NT

The Space Plasma Group will be engaged in a substantial programme of solar system exploration for the next 20 years. The work combined scientific analysis of data from AMPTE, Giotto and CRRES, with the construction of new instruments for exciting mission opportunities such as NASA Polar, Soviet Mars-94, NASA/ESA Cassini-Saturn Orbiter, NASA Cometary Rendezvous, and ESA Cluster.

There are vacancies for two post-doctoral research assistants to join the group.

1. Scientific Software Specialist.

The successful candidate will help develop flight software for the Cluster and Mars-94 missions, prepare the data analysis software for the NASA Polar project, and participate in the scientific analysis. She or he should have experience in languages like Occam (for Transputers), C or Fortran. Knowledge of plasma physics would be an advantage.

2. Space Plasma Physicist.

The successful candidate will analyse data from the group's instrument on the Chemical Release and Radiation Effects Satellite on the stability of the Van Allen belts, the generation of the aurora, and the release of Barium and Lithium. Knowledge of space plasma physics and experience in analysis of observational data is essential.

All candidates should have Ph.D. in physical science or equivalent. Starting salaries in the range £12086 to £16755. Further information is available from Dr A D Johnstone (Tel: 0483-274111) and written applications including a CV and the names of 3 referees should be sent to Prof J L Culhane, FRS, Director, Mullard Space Science Laboratory, Holmbury St. Mary, Dorking, Surrey RH5 6NT. (5806)A

UNIVERSITY OF KEELE
PROFESSOR IN THE DEPARTMENT OF BIOLOGICAL SCIENCES

We are seeking a scientist of International standing with a research background in universities or research institutes which will complement existing research groups. These range from Cellular Pharmacology, Drug Delivery, and Plant Biochemistry, to Parasitology and Ecological Entomology. Preference will be given to those using molecular or biochemical techniques to study problems at the organism level.

Salary will not be less than £27,013 per annum. **Application forms and further particulars from the Registrar, University of Keele, Keele, Staffs ST5 5BG (Tel: 0782 621111, Ext. 3008). Closing Date: 5th April, 1991.** (5821)A

TECHNICIAN
with extensive experience in animal microsurgery and cellular immunology

The Department of Surgery, University of Basel seeks applicants who are interested to work within a interdisciplinary team of surgical researchers and PhDs. The applicant should be able to independently perform standard microsurgical procedures (e.g. kidney and pancreas transplantation). In addition standard cellular immunology techniques are required.

Salary range 65'000 — 70'000 sFr. per year; travel costs will be covered.

Please reply with Curriculum vitae including two references to: Michael Heberer, M.D., Division of Research, Department of Surgery, University of Basel, Spitalstrasse 21, CH-4031 Basel, Switzerland Telephone 010 41 61 25'25'25 ext. 18-360, Fax 010 41 61 25'15'00 (W8279)A

Established Bone Marrow Transplant Program seeks an individual for appointment at the
ASSISTANT/ASSOCIATE PROFESSOR LEVEL
in the Division of Research Immunology/Bone Marrow Transplantation of the Childrens Hospital Los Angeles.

The Bone Marrow Transplant Program is involved in both the basic science and clinical aspects of bone marrow transplantation for children with immunological, oncological, hematological, and genetic diseases. The individual will be expected to engage in the clinical activities of the Bone Marrow Transplant Program and to establish an externally funded research program in the areas of immunology, hematology/oncology, genetics, or infectious disease. Individuals will receive an appropriate academic appointment in the Department of Pediatrics of the University of Southern California School of Medicine. Interested individuals should send their curriculum vitae and a summary of their research interests to **Robertson Parkman, M.D., Head, Division of Research Immunology/Bone Marrow Transplantation, Childrens Hospital Los Angeles, 4650 Sunset Boulevard, Box #62, Los Angeles, California, 90027.**

The Childrens Hospital Los Angeles and the University of Southern California School of Medicine are Equal Opportunity, Affirmative Action Employers.

(NW6504)A

INSTITUTE DIRECTOR

Applicants sought for Directorship of the James A Baker Institute for Animal Health, College of Veterinary Medicine at Cornell University. The Baker Institute has a distinguished history of accomplishment in animal health research, with continuing emphasis on studies of canine diseases. With eight senior staff, excellent laboratory and animal facilities, the Institute is well equipped for biological research. The successful applicant will have proven leadership in research and teaching and will hold faculty appointment in the College at the rank of Professor. The Director is expected to maintain an active research program. Important duties include faculty development and graduate education and support of activities which enhance public and private support of the Institute. Veterinary qualifications and/or experience in the field of animal health desirable. Send letter of interest, CV and names and addresses of three references to **Dr Fred Quimby, Department of Pathology, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853.** Review of applications will begin immediately. Cornell University is an affirmative action/equal opportunity employer. (NW6508)A

Developmental Neurobiology
POSTDOCTORAL POSITION

to study development of nervous muscle synapses. Techniques include fluorescence (including confocal) microscopy, digital image processing, histochemistry, and electrophysiology.

Send inquiries to Dr Wm Betz, Dept of Physiology, C240, Univ of Colorado Med Sch, Denver, CO 80262, USA, or to Dr RMAP Ridge, Dept of Physiology, Bristol Univ, Bristol BS8 1TD, UK. EO/AAE.

(NW6513)A

Boehringer Ingelheim

Molecular Modelling

Wir sind ein führendes Unternehmen der chemisch-pharmazeutischen Industrie. Unser Firmensitz ist in Ingelheim am Rhein, 20 Autominuten von Mainz und Wiesbaden entfernt.
Die Abteilung Pharmachemie sucht für die Gruppe Anwendung Datenverarbeitung einen/eine

Naturwissenschaftler/in

Im rahmen der computergestützten Strukturplanung in der Wirkstoffindung bieten wir Ihen ein fachlich herausforderndes Aufgabengebiet mit interessanten, konzeptionellen und anwendungsbezogenen Projektaufgaben. Diest Tätigkeit schließt auch die weiterführende Planung, Entwicklung und Integration einer abteilungsbezogenen IV-Strategie mit ein.
Die Übernahme der Position erfordert:

— sehr gute kenntnisse der theoretischen/physikalischen Chemie mit Erfahrungsschwerpunkt auf einem oder mehreren der folgenden Gebiete: Molekülmechanik, Moleküldynamik, Quantenmechanik, Konformationsanalyse von Peptiden
— gute Kenntnisse der organischen Chemie
— Erfahrungen im Umgang mit VMS- und/oder UNIX-Betriebssystemen.

Wir erwarten die Bereitschaft zur Mitarbeit in interdisziplinären Forschungsgruppen und ein ausgeprägtes Interesse an biologischen Fragestellungen.
Wir bieten Ihnen die Leistungen eines modernen Großunternehmens.
Einzelheiten möchten wir gerne in einem persönlichen Gespräch erörtern.
Zur ersten Kontaktaufnahme senden Sie uns bitte Ihre Bewerbung mit den üblichen Unterlagen.

Boehringer Ingelheim KG, Abteilung Personal/Personalbetreuung
6507 Ingelheim am Rhein. (W8301)A

UNIVERSITY OF KEELE

CHAIR IN PHARMACY PRACTICE

Applications are invited from qualified pharmacists for this new post, created by the University in collaboration with the West Midlands Regional Health Authority and the North Staffordshire Health District.

The principal responsibility of the Professor will be to establish an active research programme and, together with the Regional Pharmaceutical Office, to develop continuing education for pharmacists working in hospital and community parmacy. The Professor will also have an appropriate service responsibility with the North Staffordshire Health District.

Further particulars and application forms available from the Registrar, University of Keele, Keele, Staffs ST5 5BG, (Tel: 0782-621111 Ext. 3008). Closing Date: 5th April 1991. (5816)A

UNIVERSITY OF WARWICK

Lectureship in Organic Chemistry

A Lectureship in Organic Chemistry tenable from 1 September 1991.

Salary on the Lecturer Grade A scale
(£12,086 - £16,755 p.a.)

Application forms (returnable by 22 April 1991) and further particulars from the Personnel Office, University of Warwick, Coventry, CV4 7AL (Telephone: 0203 523627) quoting ref: 32/2A/90/14 (please mark clearly on envelope).

An equal opportunities employer. (5808)A

St George's Hospital
Medical School

University of London

NMR SPECTROSCOPIST/PHYSICIST

Cancer Research Campaign
Biomedical Magnetic Resonance Research Group

A vacancy exists for a postgraduate or postdoctoral spectroscopist or physicist to join a team developing methods for the application of Magnetic Resonance Spectroscopy, and to a lesser extent imaging, to the study and treatment of cancer.

The successful candidate will probably have a background in the physics of magnetic resonance, and ideally will have experience in its use for biological or medical research. He/she will participate in one or more research projects concerned with cancer diagnosis, tumour biology and monitoring of anticancer treatments. Expertise in computer software and radiofrequency coil building will be an advantage. The successful applicant will also manage the Group's computers (currently 3 SUN's and a NeXt).

The Group has two spectrometers: a SISCO 4.7T 200/330 and a 1.9T ORS TMR32-200; the latter is to be upgraded. Later this year the Hospital and Medical School will take delivery of a whole-body 1.5T MRI/MRS instrument. There are currently eleven members of the Group.

The salary will be up to £16,026 plus £1,767 London Allowance, subject to age and experience. The post is funded for five years from 1 January 1991. Any informal enquiries may be made to Professor J R Griffiths on 081 672 9944 extension 55994.

Further details and an application form from the Personnel Officer, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE, 081 784 2791 (24-hr answerphone). Closing date: 4th April 1991. Please quote reference 32/91.

(5838)A

Leicester
University

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL RESEARCH ASSOCIATE

Applications are invited for a postdoctoral Research Associate to work on the DNA supercoiling enzyme DNA gyrase. The Research Associate will work in the laboratory of Dr A Maxwell as part of a team working on an SERC funded project, X-ray crystallography of DNA gyrase, in close collaboration with Dr D B Wigley and Professor G Dodson of York University. Specifically the work will involve biochemical and molecular genetic experiments on DNA gyrase, including the generation and characterisation of mutant gyrase proteins, and will be aimed towards high resolution structural studies.

Candidates should have (or expect soon to have) a PhD in Biochemistry, Molecular Biology or a related discipline. The position is funded for a period of 3 years and the salary is on the RA 1A scale (£11,399-£18,165 pa).

Applicants should send a curriculum vitae and the names and addresses of two referees to Dr A Maxwell, Department of Biochemistry, University of Leicester, Leicester LE1 7RH, from whom further information can be obtained (tel: 0533 523464).

(5830)A

FACULTY POSITIONS IN BIOCHEMISTRY and CELL BIOLOGY

St Jude Children's Research Hospital

St Jude Children's Research Hospital, a private research institute, is engaged in a major expansion of its facilities and scientific activities. A new research building to be completed in early 1991 will provide 240,000 square feet of new laboratory space which will house the institute's basic science programs. As part of this expansion, new faculty positions are now available in the Departments of Biochemistry and Cell Biology. Although preference will be given to applicants who have recently completed postdoctoral training and are seeking positions at the level of Assistant Member, more senior investigators with appropriate research interests and qualifications will be considered for positions of higher faculty rank. Candidates with programs focusing in the regulation of cell growth, differentiation and transformation, the biochemistry and molecular biology of signal transduction, and mechanisms of gene regulation are particularly encouraged to apply. Opportunities exist for collaborative interactions with ongoing basic research programs in Virology and Molecular Biology, Immunology, and Pharmacology, as well as with clinical investigators in Hematology/Oncology and Infectious Diseases. In addition to new facilities and excellent start-up funds, salaries are nationally competitive and include a generous benefits package. A strong institutional commitment to its faculty ensures stability in program development and continuity of research activities.

Applicants should submit a curriculum vitae, a letter summarizing their research accomplishments and goals, and the names of three references to either:

Dr James N Ihle, or Dr Charles Sherr, St Jude Children's Research Hospital, 332 N. Lauderdale, Memphasis, TN 38015 USA.

SJCRH is an affirmative action/equal opportunity employer. (NW6457)A

INSTITUTE OF
NEUROLOGY
QUEEN SQUARE

RESEARCH ASSISTANT

Applications are invited for
three-year Research Assistar
post in the Multiple Sclerosis Sc
ciety Laboratory working o
neuroendocrine regulation
inflammatory demyelinatio
Candidates should have a back
ground in neuropharmacolog
or neuroendocrinology, and a
interest in immunology woul
be desirable. Salary on th
Grade 1A (postdoctoral) or
(postgraduate) scale up
£13,495 per annum, plus £1,76
per annum London Allowance

Applications including a C
and the names of two r
ferees should be sent to Dr
Louise Cuzner, Dept of Ne
rochemistry, Institute
Neurology, The Nation
Hospital for Neurology an
Neurosurgery, Queen Squar
London WC1N 3BG, fro
whom further informatio
may be obtained on 071 27
1338. (5820)A

CALL FOR APPLICATION

IFREMER

(Institut Français de Recherche pour l'Exploitation de la Mer)
Recruits a

SENIOR SCIENTIST

Head of its new Department of Space Oceanography Brest Center (France)

(full time appointment)

This Department of Space Oceanography (30 persons) will work in the following fields :

1 - Satellite data management beginning by ERS-1 data for the European Space Agency.
2 - Calibration and validation of satellite data.
3 - Development of basic and applied research programs making large use of active and passive satellite sensors data in the following fields : ocean circulation, climatology, air-sea interaction ans sea state, coastal oceanography and management.
4 - Application and transfer of the know how and data to economic sectors.

The Department head will have following tasks : ● Scientific and Technical Management of the department activities ● Coordination with other IFREMER departments and other research institutions and space agencies ● To advise IFREMER Directorship for its policy in space oceanography.

Candidats should have : ● High qualification : Thèse d'Etat, Ph. D. degree or equivalent ● International experience ● Experience in physical oceanography and satellite data processing ● Experience in research programe Management ● Sense of human relations ● French and english speaking

Application will include a full curriculum vitae, a description of professionnal background and research activites, the reasons of interest for application to the offered position and the name and adresses of three referees.

Applications or requests for further details should be made to M. E. CAILLIAU, **IFREMER**, DRO, 155, rue Jean-Jacques Rousseau,
92138 ISSY LES MOULINEAUX CEDEX FRANCE.

(W8308)A

PLURIAL

KARL-FRANZENS-UNIVERSITÄT GRAZ

Am Institut für experimentelle und klinische Pharmakologie der Medizinischen Fakultät der Karl-Franzens-Universität Graz gelangt ab 1. Oktober 1992 die Planstelle

einers/Ordentlichen Universitätsprofessorin/s für Pharmakologie (Nachfolge Prof Lembeck)

zur Wiederbesetzung

Gesetzliche Ernennungserfordernisse laut Anlage 1 Z 19, 1 BDG 1979 i.d.g.F. sind ein dem Aufgabenbereich entsprechendes Doktorat, eine einschlägige Lehrbefugnis und der Nachweis pädagogischer Eignung.

Von der Bewerberin/dem Bewerber wird erwartet, daß sie/er

1. imstande ist, das gesamte Fachgebiet der Pharmakologie in der Medizinerausbildung zu vertreten
2. wissenschaftlich auf dem Gebiet der Pharmakologie hervorragend ausgewiesen und im Stande ist, die Kontinuität der Forschung am Institut auf dem Gebiet der Neuropharmakologie zu gewährleisten
3. zur engen Zusammenarbeit mit international bekannten Forschungseinheiten und klinischen Disziplinen bereit ist
4. die Voraussetzungen für die Facharztausbildung garantiert
5. Fähigkeiten im modernen Management besitzt.

Bewerbungen mit den üblichen Unterlagen (Lebenslauf, Darstellung des wissenschaftlichen Werdegangs und der bisherigen Lehrtätigkeit, Publikationsverzeichnis und Nennung etwaiger anderer Qualifikationskriterien) sind unter Beischluß von zehn Separata eigener Wahl bis zum 31 Mai 1991 an den Dekan der Medizinischen Fakultät der Karl-Franzens-Universität, Universitätsplatz 3, 8010 Graz, zu richten. (W8297)A

SCIENTIST

Cancer (Tumor) Research

Development of in vitro assay to detect carcinogens of the colon with goal to screen food for components which cause colon cancer and to remove them. Development of improved animal model for human cancer using special mice implanted with patient tumors to test for optimal patient treatments and therapy evaluation. PhD in Biology or MD in Medicine and 1 year experience in job offered or as a Biologist in Cancer Research required.

Job & interview site: San Diego, CA. Salary: $32,300/yr. 40 hrs/wk. Send this ad & your resume or letter of qualifications to Job #CR4102, PO Box 9560, Sacramento, CA 95823-0560 no later than 03/29/91. (NW6514)A

THE CARDEZA FOUNDATION FOR HEMATOLOGIC RESEARCH, JEFFERSON MEDICAL COLLEGE

is seeking applicants for

POSITIONS AT THE POST-DOCTORAL AND ASSISTANT PROFESSOR LEVEL

for basic studies of endothelial cell structure and function as related to hemostasis and thrombosis. Areas of research include endothelial membrane glycoprotein synthesis, structure and function; platelet interactions with endothelial cells; and endothelial-matrix interactions. Candidates should have a working knowledge of tissue culture, membrane and protein biochemical techniques, hybridoma technology and basic techniques of molecular biology.

Postdoctoral appointments are for 2–3 years and appointments at Assistant Professor level are for 3–5 years, both renewable.

Interested applicants should submit curriculum vitae, a summary statement of their interests and names of three references to Dr S Shapiro, Director, Cardeza Foundation for Hematologic Research, Jefferson Medical College, 1015 Walnut Street, Philadelphia, PA 19107.

An Affirmative Action/Equal Opportunity Employer. (NW6511)A

COLUMBIA UNIVERSITY
Center for Radiological Research

POSTDOCTORAL RESEARCH SCIENTIST

Position available immediately for recent PhD with strong background in radiation biology, experimental pathology, or molecular genetics to work on a project involving in vitro oncogenic transformation by radiation and chemicals. Experience with human cell cultures preferable.

Send curriculum vitae, reprints and names of three references to: Dr Eric J Hall, Center for Radiological Research, College of Physicians & Surgeons, Columbia University, 630 W.168th Street, New York, NY 10032.

An Affirmative Action/Equal Opportunity Employer. (NW6505)A

THE UNIVERSITY OF BRITISH COLUMBIA

Applications and nominations are invited for the position of:

HEAD OF THE DEPARTMENT OF MICROBIOLOGY AT THE UNIVERSITY OF BRITISH COLUMBIA

The Microbiology Department, which has a long record of excellence in microbiology, immunology and molecular biology research, consists of 16 faculty members and 72 graduate students. The Department is responsible for teaching and administering a large undergraduate program. The Department is involved in collaborative teaching and research ventures with the Biotechnology Laboratory, Departments of Zoology, Botany and Oceanography and Faculties of Medicine, Dentistry and Pharmaceutical Sciences.

Research facilities include well equipped laboratories, a large scale fermentation unit and a modern animal unit. Automated DNA sequencing, DNA synthesis and protein sequencing facilities are available.

Candidates should have a record of outstanding, well funded research in microbiology or immunology, the ability to represent scientists working in diverse areas, and experience in academic administration and undergraduate teaching.

Inquiries, nominations and applications should be directed to the **Search Committee Chairman, Dr Barry C McBride, Faculty of Science, 6270 University Boulevard, Vancouver, BC, Canada, V6T 1W5.** Closing date for applications is May 30, 1991. Salary will be commensurate with qualifications and experience. In accordance with Canadian immigration requirements priority will be given to Canadian citizens and permanent residents of Canada. The University of British Columbia encourages qualified women and minority applicants.

(NW6521)A

The University of Waikato

Hamilton, New Zealand

LECTURESHIP IN CHEMISTRY

Ref. no. A90/113

The University has a vacancy for a Lecturer in Chemistry. Applicants should have a PhD or equivalent and postdoctoral experience in research and/or teaching. Applications are invited from chemists with interests in any branch of chemistry, but will be especially welcome from persons with expertise in one or more of the following areas: Physical Chemistry of Biological Systems; Surface Chemistry and Catalysis; Industrial/Applied Chemistry; Mass Spectrometry; Inorganic Chemistry.

The successful candidate will be expected to teach at undergraduate and graduate levels, to supervise MSc and DPhil students, and to pursue research.

The salary range for Lecturers is currently: NZ$37,440-NZ$49,088 per annum.

Enquiries of an academic nature may be made to the Chairperson of Chemistry, Associate Professor D W Smith, tel (64) 71 562889, Fax (64) 71 560115. Information on the method of application and conditions of appointment can be obtained from the **Academic Staff Unit, University of Waikato, Private Bag 3105, Hamilton, New Zealand (tel. (64) 71 562 889, Fax (64) 71 560135). Applications quoting reference number A90/113 should reach the Academic Staff Unit by 30 April 1991.**

Places for appointees' children may be available in the creche run by the Campus Creche Society (Inc). The University welcomes applications from suitable people regardless of race, creed, marital status or disability.

(W8303)A

DEPARTMENT OF BIOCHEMISTRY UNIVERSITY OF OXFORD

OXFORD GLYCOBIOLOGY INSTITUTE RESEARCH ASSISTANTS/ SENIOR SCIENTISTS BR/354

The Oxford Glycobiology Institute programme seeks to identify how carbohydrates modify protein structure and function and how they are involved in recognition events. The Institute has a programme of technical development which involves the isolation sequencing and conformational studies of oligosaccharides, together with supporting biochemical techniques which are useful in glycobiology. The Institute is seeking to appoint at least two Research Assistants/Senior Scientists. Applicants should have at least two years of relevant post-doctoral experience in one of these fields and be keen to build up a team which will study the glycobiological aspects of these areas within the context of the main programme of the Institute. There will be the option of participating in the teaching programme within the Department of Biochemistry.

Salary will be on the RS1A Scale £11,399 - £18,165 per annum, depending upon age and experience, consideration may be given to appointing at RS11 if appropriate.

The apointment will be for five years with the possibility of renewal.

Applications, which should include a full curriculum vitae, together with the names and addresses of two or three referees should be sent to:

The Senior Administrator, Dept of Biochemistry, South Parks Road, Oxford OX1 1QU.

AN EQUAL OPPORTUNITY EMPLOYER. (5801)A

DEPT OF BIOCHEMISTRY, SOUTH PARKS ROAD, OXFORD OX1 1QU

BRUNEL UNIVERSITY

Department of Biology and Biochemistry

(Post ref: P727/R)

POST DOCTORAL RESEARCH ASSOCIATE

to work with Professor T F Slater and DR K H Cheeseman on a study into oxidative stress and free radical reactions in relation to the growth of cells in culture. This is a new project under the auspices of the Animal Cell Club of the SERC Biotechnology Directorate. The techniques involved will include cell culture in both flasks and a small-scale bio-reactor and determinations of antioxidant capacity and indices of oxidative stress in cultured cells by techniques including h.p.l.c. Experience in cell culture is essential and an interest in free radical biochemistry would be desirable. The appointment is available from 1 April 1991 for a period of three years.

Salary within the Research 1A scale up to £15,262 per annum inclusive of London Allowance.

(Post ref: P727/T)

TECHNICIAN

to work on the research project described above. Experience of cell culture techniques would be an advantage but not essential. The appointment, is available from 1 April 1991 for a period of three years.

Salary within the Technician grade C scale up to £9,675 per annum inclusive of London Allowance.

For further information please contact Dr K H Cheeseman (Tel: 0895 74000 ext 2086).

Application forms can be obtained from the Personnel Office, Brunel University, Uxbridge, Middlesex UB8 3PH or telephone 0895 812304 (24 hours) leaving your name, address, and post reference. Closing date: 4th April, 1991. (5835)A

MOLECULAR EVOLUTIONARY BIOLOGIST

The Department of Ecology and Evolutionary Biology at Princeton University plans to appoint a molecular evolutionary biologist. Rank is open. The appointment can begin any time between July 1991 and September 1992. The closing date for applications is April 15, 1991.

Applicants should send a curriculum vitae and arrange for three letters of reference to be sent to Henry S Horn, Search Committee, Department of Ecology and Evolutionary Biology, Guyot Hall, Princeton University, Princeton, NJ, 08544-1003.

Princeton University is an equal opportunity/affirmative action employer.

(NW6509)A

UNIVERSITY OF LONDON
Wye College
POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for a Postdoctoral Research Assistant funded for two years by the Leverhulme Trust to work on "Biosynthesis and Significance of Lipid Conjugates of Xenobiotic Acids in Plants". The person appointed will have or will be due shortly to complete a Ph.D. and experience of one or more of the following, though not essential, would be an advantage: plant cell fractionation, lipid biochemistry, metabolism of xenobiotic compounds. He or she will join a laboratory active in lipid biochemistry and with an established record in the field of xenobiotic lipids in animal systems.

The appointment will be on the 1A scale for Research and Analogous Staff at an initial salary of up to £13,495 (the 4th point of the scale).

Applications by letter, together with a cv including the names and addresses of two academic referees should be sent to the Dr T A Hill, Assistant Director of Administration, Wye College, (University of London), Wye, Ashford, Kent TN25 5AH Telephone: 0233 812401 Extn. 228. Informal enquiries may be made by telephoning Dr P F Dodds at the above number, Extn. 469.

Wye College is an Equal Opportunities Employer. (5815)A

NEUROCHEMIST
MEDICAL RESEARCH COUNCIL
Unit of Clinical Pharmacology, Oxford

A neurochemist is required for the investigation of the mechanisms by which psychotropic drugs affect 5-HT function. Applicants should have post-doctoral experience and be capable of creative and independent work. Preference will be given to those who have worked previously on intra-neuronal second messenger systems. An added advantage would be a knowledge of ion channel function in relationship to neurotransmission at either the pre- or post-synaptic level.

The MRC Unit has both basic and clinical sections of Psychopharmacology, and is closely associated with the Unversity Department of Clinical Pharmacology and the SmithKline Beecham Centre for Applied Neuropsychobiology. The successful applicant will work in a multi-disciplinary neuroscience environment, and will be responsible for the neurochemical investigations in the MRC Unit.

Please send application, CV and list of publications to Professor D G Grahame-Smith, MRC Unit of Clinical Pharmacology, Radcliffe Infirmary, Oxford OX2 6HE. Closing date for applications: 5th April, 1991. (5823)A

THE LONDON HOSPITAL MEDICAL COLLEGE
(University of London)
Molecular Biology
POST-DOCTORAL RESEARCH FELLOW
Medical Unit

A three year position, funded by the British Diabetic Association, to investigate the immungenetics of diabetes mellitus is available immediately. The successful candidate will join our established molecular biology group. Experience in the use of the polymerase chain reaction and direct DNA sequencing would be advantageous.

The salary is on the Research Fellow 1A scale, starting at £13,495 per annum (according to experience), plus £1,767 London Allowance.

Further details may be obtained from Dr Graham A Hitman. Tel: 071 377 7111/Fax: 071 377 7677.

Applications, including curriculum vitae and the names of two referees, should be sent to the College Secretary, The London Hospital Medical College, Turner Street, London E1 2AD.

Closing date: 4th April, 1991. (5834)A

MRC Dental Research Unit
Periodontal Diseases Programme
The Medical College of The Royal London Hospital
Post-Doctoral Research Opportunities
Molecular Biologist & Biochemist

£14,148 — £22,226 (inclusive of London Weighting)

The Molecular Biologist

*Interest in Microbial pathogencity
*To work on genetic analysis of oral anaerobes
*Experience in PCR, cloning and sequencing cloned products
*To lead small well-resourced team
*3-year appointment

The Biochemist

*Interest in Protein chemistry
*To study the functions of immunodominant antigens of periodontal pathogens
*To join a small, prolific and well-resourced team
*3-year appointment

The Unit

*Small, enthusiastic, productive, closely-knit
*Well resourced with MRC and Industrial backing
*An integral part of the research community of a dynamic Medical College
*Good links with other international centres of excellence

The Contacts

*Roy Gillett on 071 377 0444 for further information and informal visits
*Professor Newell Johnson, Director, for formal applications (to include full c.v and names of two referees) MRC Dental Research Unit, 32, Newark Street, London, E1 2AA.

The Deadlines

*Closing date 28th March 1991
*Interview date 5th April 1991

Be in touch to find out more.

(5825)A

MEDICAL RESEARCH COUNCIL
Dunn Nutritional Laboratory
POST-DOCTORAL RESEARCH WORKER

A post-doctoral (PhD) research worker post funded by Nestle, Switzerland, is available immediately for a three year project to study the antioxidant properties of lutein and some of the other major xanthopyll carotenoids in blood and tissue for potentially important roles of physiological significance in health and disease. Applicants should have research experience in free radical mechanisms in biochemistry or organic chemistry and an interest in the influence of nutrition on disease.

For further information please contact Dr. David Thurnham (0223 426356).

We expect to appoint at a salary in the region of £14,000 to £16,000 per annum, depending on qualifications and experience. Applications (two copies) including a curriculum vitae and the names and addresses of two professional referees should be sent to the Unit Administrator, Mrs. E. Millar, MRC Dunn Nutritional Laboratories, Downhams Lane, Milton Road, Cambridge CB4 1XJ quoting reference: DT/NESTEC1.

The closing date for applications is 29th March 1991. The Medical Research Council is an Equal Opportunities Employer. (5814)A

CAREER SCIENTIST

(tenure-track faculty) position with primary appointment in the Department of Anesthesiology and possible joint appointment in a basic science department. Candidate should possess a Ph.D., M.D., or equivalent degree with postdoctoral experience and demonstrated ability to attract extramural research funding. Primary research interests should be in the areas of molecular pharmacology, physiology, or biochemistry with direct or indirect applicability to anesthesiology, critical care medicine or pain management. Generous laboratory space and startup funds provided. Academic rank will be determined by qualifications. Established investigators are especially encouraged to apply. **Please send a description of research interests, curriculum vitae, names and phone numbers of three references to P. R. Housmans, M.D., Ph.D., Mayo Medical School, Rochester, MN 55905 by May 1, 1991.** *Mayo Foundation is an affirmative action and equal opportunity educator and employer.* (NW6475)A

PALM OIL RESEARCH INSTITUTE OF MALAYSIA (PORIM)

SENIOR RESEARCH FELLOW (TISSUE CULTURE)

Applications are invited for the post of Senior Research Fellow (Tissue Culture) from candidates with PhD trained in biochemistry or molecular biology in relation to plant morphogenesis. Experience in tissue culture induced variations would be desirable.

The successful candidate will be a member of a tissue culture team and will work together with technical staff and assistants.

It is envisaged that the initial appointment would be for a period of one to three years. The salary will be based on experience but will not be less than M$54,000 per annum with two months' gratuity for every completed year. A fixed entertainment allowance of M$9,600 per annum is payable. Accommodation will be provided.

Information on the proposed appointment, terms and conditions would be made available on request.

Applications, including (i) full curriculum vitae (ii) brief proposal of research the candidate wishes to pursue in PORIM (iii) non-returnable photograph and (iv) names of three referees, are to reach Director-General, PORIM, PO Box 10620, 50720 Kuala Lumpur, Malaysia. Applications will be accepted until a suitable candidate is found. (W8305)A

Prifysgol Cymru, Bangor, University of Wales and Rivm Bilthoven, The Netherlands

School of Biological Sciences

Microbiologists/Biochemists/ Molecular Biologists

2 Year Contracts

Post Doctoral Workers – Salary:- £11,399 – £18,165 per annum

Technicians Scale C – Salary:- £8,405 – £9,424 per annum

Four posts are available (two post-doctorals and two technicians) to work on a two-year project funded by the European Community. One **Postdoctoral Worker** and one **Technician** will be based in the Netherlands. These will be paid on the nearest Dutch equivalent salaries. The project involves investigation of soil bacteria containing catabolic plasmids that are important in the biodegradation of xenobiotics. Techniques will include genetic and biochemical characterisation of isolated bacterial strains and genetic engineering of catabolic plasmids (posts based in Bangor) and competition of bacterial strains in chemostat culture (posts based in Bilthoven, The Netherlands).

Applications (two copies), giving details of qualifications and experience together with the names and addresses of two referees should be sent to **Mr. Alan James, Personnel Officer, University College of North Wales, Bangor, Gwynedd LL57 2DG,** by not later than **8th April, 1991.**

An Equal Opportunities Employer (5843)A

POSTDOCTORAL POSITION

to examine the regulatory role of phosphorylation on the function and metabolism of tau and other neuronal cytoskeletal proteins. Protein biochemistry experience is desirable.

Send CV and the names of 3 references to: Dr Gail V W Johnson, Dept of Cell Biology, Sparks Center 911, Univ Alabama at Birmingham, Birmingham, AL, USA 35294-0017. FAX# (205)-934-3709.

(NW6518)A

EXECUTIVE DIRECTOR

To head established wildlife conservation organization, Philadelphia based, staff of four. Opportunity to lead US membership organization working with Gerald Durrell's Jersey Wildlife Preservation Trust. Position involves planning development, administration (including field projects), communications, Board liaison, some travel. Salary commensurate with experience. Equal opportunity employer.

Send resume including salary history and availability to: Dr Charles H Watts, Wildlife Preservation Trust International, 34th St and Girard Avenue, Phila, PA 19104.

(NW6510)A

DEPARTMENT OF BIOCHEMISTRY UNIVERSITY OF OXFORD

WELCOME TRUST POSTDOCTORAL RESEARCH ASSISTANT IN BACTERIAL SENSORY TRANSDUCTION BR/357

Applications are invited for a postdoctoral research assistant to join a group investigating motility and behaviour in bacteria. The investigation involves molecular biology and bioenergetics in combination with computerized motion analysis, therefore scientists with interests in any of these areas are invited to apply. This project specifically involves the investigation of role of two-component sensory systems in controlling both bacterial behaviour and gene transcription and the mechanisms of integration of these sensory signals.

The position, which is funded by the Wellcome Trust, is available immediately for three years.

Salary is on the 1A Scale £11,399 – £18,165 per annum depending upon age and experience. The starting salary will include additional increments in line with Wellcome Trust policy.

Informal enquiries to Dr. J. P. Armitage (0865-275299).

Applications, including a full c.v. and the names and addresses of two referees should be sent to:

The Senior Administrator

AN EQUAL OPPORTUNITY EMPLOYER. (5804)A

DEPT OF BIOCHEMISTRY, SOUTH PARKS ROAD, OXFORD OX1 1QU

ENDOWED CHAIR IN BIOLOGICAL CONTROL

The Department of Entomology and Nematology, IFAS, University of Florida, invites applications and nominations for the newly-established Davies, Fischer and Eckes Chair in Biological Control. The eminent scholar will develop innovative research programs on biological control of arthropods, nematodes, and or weeds. His/her research may be fundadamental or applied, and may focus on model systems, beneficial organisms, pest complexes, or other biological processes. It is anticipated that the research results generated by this position will benefit the citrus industry. The incumbent will interact with biocontrol faculty statewide, and develop a strong graduate program, including instruction. Candidates are expected to be internationally recognized scholars, possess a doctorate, have a proven record of obtaining extramural financial support, and display a strong commitment to graduate education.

Applicants must submit, by 1 June 1991 a curriculum vitae, statement of research interests, and names of five (5) references to John L Capinera, Chairman, Dept Entomology & Nematology, Building 970, Hull Road, University of Florida, Gainesville, Florida 32611-0740.

The University of Florida is an Equal Opportunity/ Affirmative Action Employer. (NW6519)A

SIR WILLIAM DUNN SCHOOL OF PATHOLOGY UNIVERSITY OF OXFORD

POSTDOCTORAL IMMUNOLOGIST

Salary

£11,399-18,165

A postdoctoral position is available on an MRC Programme Grant to join a group researching into the properties and functions of stromal macrophages in murine lymphohaemopoietic tissues. The work will focus on novel non-phagocytic cell surface receptors involved in cell interactions. A background in cellular immunology or immunochemistry is required. The post is available until 31 December 1993.

Informal enquiries may be made to Professor S Gordon (tel: 0865 275534).

Applications in writing, together with cv and names and addresses of two referees, should be sent to the Administrator, Sir William Dunn School of Pathology, South Parks Road, Oxford OX1 3RE. Closing date for applications is 14 April 1991.

The University is an equal opportunity employer. (5829)A

POSTDOCTORAL RESEARCH ASSOCIATE

To work on a quantitative, neural network theory of hippocampal function. Should have a background in theoretical analysis using probability and entropy measures, neural network experience desirable but not necessary. Position funded for 5 years, available Summer 1991. Salary negotiable.

Send CV and three references to W B Levy, Neurosurgery Research, Univ of Virginia, Health Sciences Center, Box 420, Charlottesville, VA 22908. EOAAE. (NW6517)A

UNIVERSITY OF CAMBRIDGE

ASSISTANT LECTURESHIP IN PETROLOGY

Applications invited in any area of Petrology, including experimental, theoretical or field based approaches to the subject.

Salary scale: £12,000-£16,800.

Applications, including cv (10 copies) and names of three referees, to be sent to the Administrator, Department of Earth Sciences, Downing Street, Cambridge CB2 3EQ, UK by 3 May 1991. (5827)A

OVER HALF A MILLION SCIENTISTS ARE LOOKING AT THIS SPACE.

nature

SHOULDN'T YOU BE FILLING IT?

MEDIZINISCHE
HOCHSCHULE
HANNOVER

MHH

Institute of Molecular Biology

Applications are invited for an

ENDOWED PROFESSORSHIP

(C3-Professur)

in the Institute for Molecular Biology, Hannover Medical School, Hannover, Federal Republic of Germany. Final financial approval for this position is pending from the local government of Niedersachsen. The position will be a permanent one funded by the Volkswagen-Stiftung to carry out research on the topic:

"Molecular Biology of Cellular Ageing"

Research should address the problems of cellular senescence and regulation of cell proliferation. In this connection, questions of the regulation of the cell cycle, the role of tumor suppressor genes, and aspects of cellular immortalisation, differentiation and oncogenesis are important. A feasible alternative would be the investigation of the molecular causes of human age-related illnesses (dementia, etc.) or of multiple sclerosis.

The successful applicant will have attained „Habilitation" or an equivalent qualification (in accordance with § 56 of the "Niedersächsische Hochschulgesetz"). Applications enclosing an outline of research plans and a c.v. should be sent to:

Rektor der Medizinischen Hochschule Hannover
Konstanty-Gutschow-Str. 8 · 3000 Hannover 61
Federal Republic of Germany

The deadline for receipt of applications is May 31, 1991. (W8299)A

UNIVERSITY OF BRITISH COLUMBIA

HEAD, DEPARTMENT OF PHYSIOLOGY

The Faculty of Medicine, University of British Columbia invites applications and nominations for the position of Head of the Department of Physiology.

We seek an academic leader to be responsible for directing and developing the teaching and research programs of the Department. The Department has 18 Full-time Faculty members and attracts research support of $1,840,000 annually. Candidates should have a proven record of scholarly activity, a strong research background and a commitment to undergraduate and graduate medical education. Starting date will be July 1, 1991. Salary will be commensurate with experience and qualifications.

UBC encourages qualified women and minority applicants. In accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents.

Applications, accompanied by a detailed curriculum vitae and the names of three references, should be directed by April 15, 1991 to:

Dr M J Hollenberg
Dean, Faculty of Medicine
Room 317, Instructional Resources Centre
University of British Columbia
2194 Health Sciences Mall
Vancouver, BC V6T 1W5.

(NW6522)A

POSTDOCTORAL ASSOCIATE POSITION

ION CHANNELS IN ARTERIAL SMOOTH MUSCLE

UNIVERSITY OF VERMONT

This position will involve the investigation of the modulation of calcium and potassium channels in cerebral and coronary arteries by vasoactive substances (neuropeptides, transmitters, and endothelial factors), by vasodilating drugs, and by cellular metabolism. Available techniques include arterial diameter and membrane potential measurements, single channel and whole cell current measurements in isolated single cells and in bilayers, and free calcium measurements. A competitive salary with full benefits is offered.

Applicants should send CV and names of references to: **Dr M Nelson, Dept of Pharmacology, University of Vermont, Burlington, VT 05405-0068. Fax: (802) 656-4523.**

(NW6524)A

POSTDOCTORAL RESEARCH POSITION

Receptor biochemistry of brain in prion diseases. Salary supported by NIH training grant requires US citizenship or permanent resident visa.

Send curriculum vitae and three letters of recommendation to: Stephen J DeArmond, Neuropathology, University of California, San Francisco, CA 94143-0506. (NW6512)A

UNIVERSITY OF BRISTOL
Department of Biochemistry

RESEARCH ASSISTANT

Applications are sought for a three-year position funded by the MRC to study the mechanism of transmembrane signalling by the human insulin receptor. The work will involve the use of site-directed and deletion mutagenesis of the human insulin receptor cDNA and its expression in a variety of cultured cell lines. Experience in molecular biology and/or tissue culture would be an advantage.

The position is funded at the research assistant 1A or 1B level and carries a salary of up to £12,086 per annum with possible registration for a PhD at the 1B level.

Informal enquiries may be made to Dr JM Tavare on Bristol (0272) 303030 ext 4961/4962.

For further details telephone Bristol 256450 (ansaphone after 5.00pm) or write to the Personnel Office, Senate House, Bristol BS8 1TH. Please quote reference 623.

An Equal Opportunities Employer. (5824)A

UNIVERSITY OF OXFORD

PHOTOELECTRON MICROSCOPY
Post Doctoral Research Assistantship

The Photoelectron SpectroMicroscopy group directed by Professor D.W.Turner FRS expects shortly to install a new instrument of advanced design. PESM is a novel technique that enables very sensitive surface analysis at high resolution. This is a collaborative project with Kratos Analytical Ltd. under a DTI/SERC link programme. The new instrument is directed towards real-time imaging of changes in surface chemistry and will offer possibilities for simultaneous surface studies with different ionization sources and selected energy electron imaging from thermal to soft X-ray energies. Applications are invited from experimental physicists or physical chemists for a new appointment at the Postdoctoral level to support this work. Experience in XPS or other surface analysis techniques, particle beams, or Ultra High Vacuum will be expected. The appointment will be within the 1A range £11,399 - £18,165. It covers a three year period and would be made for one year in the first instance.. Requests for further information or applications including C.V. and the names of two referees should be directed to: **Professor D.W. Turner, Physical Chemistry Laboratory, South Parks Road, Oxford OX1 3QZ.**

(5845)A *The University is an Equal Opportunity Employer* KRATOS

Immunogenetics Research Foundation
$100,000
Sequence Analysis
m-Ab Production

The Immunogenetics Research Foundation is providing $100,000 per annum for at least three years to support a DNA and Peptide Sequence Analyst who would join a group supported by a NH&MRC Programme Grant "Immunogenetics and Autoimmune Disease". New MHC genes have been sequenced and mapped and it is now intended to recruit an appropriate team leader to direct the activities of several programmers (DNA and protein sequence analysis) and technicians (monoclonal antibodies against putative epitopes). The resulting antibodies would then be used by Cellular Immunologists to study the expression and function of potentially relevant genes.

$A100,000 (US$78,150) is available for a package including salary, travel, and programme and secretarial support. Excellent facilities including an OS/2 local area network with 40 PC workstations are available.

For further expressions of interest please contact R L Dawkins - Department of Clinical Immunology, Royal Perth Hospital, Box X2213, GPO, Perth WA 6001, Australia. Phone 61-9-224 2899 or fax 61-9-224 2920.

(W8302)A

NEW OPPORTUNITIES FOR MOLECULAR GENETICISTS

The Center for Human Genetics at Boston University School of Medicine now offers two new positions for PhD Molecular Geneticists at the Assistant or Associate Professor rank. Established investigators preferred with expertise in gene mapping, cloning, in situ hybridization, sequencing, immunogenetics, or use of novel techniques applicable to complex genomes. Interaction with over 20 Molecular Biologists/Geneticists in other departments and many more in Boston.

Send CV's to: Aubrey Milunsky, MD, DSc, Director, Center for Human Genetics, Boston University School of Medicine, 80 East Concord Street, Boston, MA 02118. BUSM is an equal opportunity/affirmative action employer.

(NW6525)A

We
guarantee to
publish
your
advertisement
on Thursday
if received
by noon
the previous
Friday

THE AUSTRALIAN NATIONAL UNIVERSITY
THE JOHN CURTIN SCHOOL OF MEDICAL RESEARCH
DIVISION OF BIOCHEMISTRY AND MOLECULAR BIOLOGY
POSTDOCTORAL FELLOW PROTEIN BIOCHEMISTRY GROUP

A position is available for a Pos doctoral Fellow, to work in coll boration with Dr Michael Denb rough on the biochemistry an molecular biology of the musc membrane disorder which predi poses to death during anaesthes (malignant hyperpyrexia), hea stroke and cot death. Experience protein biochemistry and/or mol cular biology would be an advan age.

Enquiries to Dr Denboroug (tel.(61 6) 249 2499).

Previous applicants (who r sponded to advertisement J 10.10.1) remain under conside ation.

This is an externally-funded pos tion and is available for two yea only.

Closing date: 5 April 1991. Re JC 26.2.1.

Salary: Postdoctoral Fello Grade 1 (fixed point) A$28,79 A$32,762 pa.

Applications should be submitte in duplicate to the Registrar, Th Australian National Universit GPO Box 4, Canberra ACT 260 Australia, quoting reference num ber and including curriculum vita list of publications and names of least three referees. The Universit reserves the right not to make an a pointment or to appoint by invit tion at any time. Further inform tion is available from the Registra or from Appointments (39151 Association of Commonweal Universities, 36 Gordon Squar London WC1H 0PF.

The University is an equal oppo tunity employer. (W8306)A

CANADIAN INSTITUTE FOR ADVANCED RESEARCH AT UBC
POSTDOCTORAL CELL OR MOLECULAR BIOLOGISTS

Two positions for recent PhDs to study gene structure and organelle biogenesis in Archezoa (primitively amitochondrial eukaryotes eg *Giardia*), using PCR, cloning, automated DNA sequencing, and immunocytochemistry. We encourage qualified women and minorities.

Send cv, summary of research interests, and names/fax/phone numbers of 3 referees to: **Dr T Cavalier-Smith (tel (604) 822-4906, fax (604) 822-6089), Department of Botany, University of British Columbia, #3529-6270 University Blvd, Vancouver, BC, Canada V6T 1Z4.** (NW6523)A

THE INTERNATIONAL RICE RESEARCH INSTITUTE

Position Announcement for

PLANT MOLECULAR GENETICIST

The International Rice Research Institute is seeking a Plant Molecular Geneticist to be based in Los Banos, Laguna, Philippines, to work on genome mapping and marker-based selection for rice improvement.

The International Rice Research Institute (IRRI) is an international, non-profit, donor-funded, agricultural research institute that undertakes research and training at its headquarters in the Philippines and collaborates with national agricultural systems worldwide. Its goal is to develop more productive and sustainable rice technology and to strengthen national research capability.

The objectives of the Institute's biotechnology research are to produce novel rice germplasm through alien gene transfer, to evaluate the genetic variability of world rice germplasm and related pests, to study the genetics of agronomically important characters based on RFLP and isozyme analysis, and to increase the efficiency of breeding and pest management through the use of marker-aided selection and the production of another-culture-derived doubled haploid lines, and molecular diagnostic techniques.

Responsibilities: Work as a member of an interdisciplinary team of plant breeders, pathologists, entomologists, and physiologists to tag genes of agronomic importance via linkage with RFLP and isozyme markers, with emphasis on quantitative traits. Develop a research program in molecular genetics aimed at incorporating molecular market technology into the plant breeding program. Collaborate with advanced molecular biology laboratories to develop the potential for map-based gene-cloning and transformation of rice. Contribute to degree and non-degree biotechnology training for National Rice Research Systems. International travel will be necessary.

Qualifications: PhD or equivalent in Plant Molecular Biology, Plant Breeding, Genetics, Biochemistry, or related discipline. Experience in RFLP mapping and quantitative trait analysis, and familiarity with plant selection strategies. Demonstrated skill in interdisciplinary research and in communicating with international teams of plant breeders and other scientists. Skills in project leadership and previous participation in rice biotechnology training would be advantage.

Benefits: Salary and perquisites are competitive with those of CG and other international institutes in agriculture.

Applications: Full documentation, with names of three referees and indicating date of availability should be sent to:

Dr Klaus Lampe, Director General
IRRI, PO Box 933, 1099 Manila, Philippines
Fax: 63-2-818-2087. Telex: 40890 RICE PM or 22456 IRI PH.

(W8312)A

STOP PRESS!

Due to the Easter recess our classified deadline for the April 4th issue will change as follows:

UK., EUROPE, REST OF WORLD:	Tel: (071) 872 0102	Fax (071) 240 2408
FRANCE:	Tel: (1) 48 87 24 43	Fax: (1) 48 87 46 71
GERMANY/AUSTRIA:	Tel: (089) 52 40 82	Fax: (089) 5 23 22 22
JAPAN:	Tel: (03) 3267 8751	Fax: (03) 3267 8746

All material must be received by March 27th, 5.00 pm Local time.*

NORTH AMERICA

South/Mid West, North East:	Tel: (212) 477 9625	Fax: (212) 505 1364

All material must be received by March 26, 1991.*

Western States:	Tel: (415) 781 3805	Fax: (415) 781 3805

All material must be received by March 25th, 1991.*

Canada:	Tel: (416) 868 1213	Fax: (416) 868 1213

All material must be received by March 25, 1991.*

***Please contact your nearest sales office for the absolute latest deadlines!**

Post-doctoral Scientists

Applied Development Laboratory – London

The Imperial Cancer Research Fund is one of the largest charity research institutions in Britain with wide ranging interests in fundamental, applied and clinical research.

Applications are invited from post-doctoral scientists to work on a directed programme of research involving genetic engineering of monoclonal antibodies with potential clinical application. This will include humanisation of antibodies, construction of chimeric antibodies and selection of antibody activities from various expression libraries. Initially the scientist appointed will be working on the cloning and characterisation of an antibody with potential in the diagnosis and therapy of colon cancer.

It is expected that candidates will have demonstrated achievements in molecular genetics and, ideally, in the area of genetic engineering. The appointments will be for three years in the first instance with possible renewal for a further three years during which time suitable candidates may be considered for a tenured appointment.

The Applied Development Laboratory will be located in the ICRF Laboratory at Dominion House, St Bartholomew's Hospital. Interaction with the ICRF central laboratory in Lincoln's Inn Fields is encouraged and access to central resources will be provided.

Starting salary will be in range of £18,272–£21,784 although a higher salary will be available for an exceptional candidate.

(5840)A

Application should be made by sending full Curriculum Vitae and names and addresses of three referees to the Recruitment Office, Imperial Cancer Research Fund, PO Box 123, Lincoln's Inn Fields, London WC2A 3PX, quoting Ref: 12/R. Closing date: 12 April 1991

SMOKING IS ACTIVELY DISCOURAGED

Imperial Cancer Research Fund

FELLOWSHIPS/STUDENTSHIPS

20 FELLOWSHIPS IN PLANT MOLECULAR BIOLOGY

Available from October 1, 1991

Deadline for application May 1, 1991

The Training Network on Applications of Genetic Engineering and Molecular Biology for Seed Improvement invites applications for junior or senior fellowships.

Conditions:

The postgraduate research has to be carried out in an EEC country different from the fellow's present resident country.

Only residents in an EEC country are qualified.

For application forms and information on the laboratories participating in the Network write to:

Professor Diter von Wettstein
Carlsberg Laboratory
Gamle Carlsberg Vej 10
DK-2500 Copenhagen Valby
Denmark
Telefax: +45 31 21 13 66

(W8300)E

Post-Doctoral Fellowships

The Imperial Cancer Research Fund is one of the largest independent cancer research institutes in Europe employing approximately 450 scientists and clinicians. It has a wide-ranging programme in fundamental, applied and clinical cancer research.

There are approximately seventy research groups encompassing the major disciplines of cellular and molecular biology including biochemistry, genetics, immunology, and virology. Most are housed at the main building in Central London: there are also research units at Clare Hall, North London and at Dominion House attached to St. Bartholomew's Hospital. ICRF's external clinical units based in major teaching hospitals form the link between fundamental research and clinical activities.

Each research group works independently. All have access to centralized services including cell production, oligonucleotide and peptide synthesis, amino acid and DNA sequencing, a library of DNA probes and somatic cell hybrids for gene mapping, experimental animal breeding and maintenance including transgenic mice, electron microscopy, and a large computer facility.

Fellowships

Approximately 40 post-doctoral fellowships are available each year to scientists of any nationality. Most of these are three-year fellowships, but visiting fellowships for a period of one or two years are also offered.

Starting salaries are in the range £16,286-£19,610 (Central London), £15,446-£18,769 (Outer London) and £14,605-£17,929 (Cambridge), dependent on age and experience. This is subject to UK tax but avoidance of double taxation agreements exist with all countries of the EEC, the Commonwealth and the USA. Visitors are entitled to medical and dental care under the National Health Service. ICRF will provide assistance with relocation expenses.

The projects currently available are listed below.

1. **Stephan Beck**: Development of chemiluminescent detection chemistries for DNA sequencing.
2. **David Bentley**: Control of c-*myc* transcription by attenuation
3. **Lionel Crawford**: The role of cell mediated immunity in human papillomavirus associated tumours.
4. **Paul Freemont**: X-ray crystallographic studies of DNA and calcium binding proteins.
5. **Michael Fried**: Molecular mechanisms involved in gene expression.
6. **Ian Hart**: Role of glycoprotein CD44 in metastatic behaviour of melanoma cells.
7. **Hartmut Land**: Cooperating oncogenes and cell cycle regulation.
8. **Michael Owen**: The role of the Ets family of transcription factors in T cell differentiation and activation.
9. **Malcolm Parker**: Mechanisms of transcriptional regulation by steroid hormones.
10. **Gordon Peters**: Oncogenes on chromosome 11q13 in human breast cancer.
11. **Enrique Rozengurt**: Early signals in cell proliferation.
12. **Joyce Taylor-Papadimitriou**: Analysis of molecular events in the expression and processing of a tumour antigen expressed by breast cancers.
13. **John Trowsdale**: Function and regulation of novel human MHC genes.
14. **Stephen West**: The molecular biology of genetic recombination.
15. **Nick Wright and Susan Kirkland**: Control of differentiation and heterogeneity in colorectal carcinoma.
16. **Bryan Young and Suzanne Watt**: Functional analysis of stem cell proteins.

(5805)E

For further information and application form, please write to the Recruitment Officer,
Imperial Cancer Research Fund, PO Box 123,
Lincoln's Inn Fields,
London WC2A 3PX.
Tel 071-242 0200 ext 3357,
quoting reference 53/R.

SMOKING IS ACTIVELY DISCOURAGED.

Imperial
Cancer
Research
Fund

MOLECULAR GENETICS

A post-doctoral fellowship position is available immediately to study the molecular genetics of pediatric brain tumors. Initial research will focus on loss of heterozygosity on chromosomes 17p and 22q and PCR based sequencing of p53. Experience in molecular biology is required. Excellent working environment in new laboratory space at The Children's Hospital of Philadelphia, on The University of Pennsylvania campus.

Send letter, CV and names of three references to Dr Jaclyn Biegel or Dr Beverly Emanuel, Division of Human Genetics and Molecular Biology, The Children's Hospital of Philadelphia, 34th St and Civic Center Blvd, Philadelphia, PA 19104 or Fax to 215-590-3850.

(NW6520)E

---

nature

the widest
international
selection of jobs
in science
— EVERY WEEK

Post Doctoral Research Fellowship

Cellular differentiation in bone formation

ICI Pharmaceuticals is one of ICI's major international businesses ...with an outstanding record of research and development . Our commitment to scientific excellence and innovative research has already resulted in market leading products which include anti-infectives, anaesthetics, anti-cancer agents and cardiovascular drugs.

Operating at the leading edge of technology provides an exciting challenge for the scientists working with us. As part of our continued commitment to high level research, we have created a new Post Doctoral Research Fellowship. This is an excellent opportunity for a young PhD scientist to work alongside experienced molecular biologists within our Biotechnology Department. You will gain considerable technical expertise in bone cell biology through collaboration with a leading expert in the bone field, Dr Brian Ashton at the Institute of Orthopaedics, Oswestry. The objective of the research will be to analyse osteoblast differentiation and factors which control this process using molecular biology techniques.

To join our Bone Disorders research team, candidates are likely to be in their mid to late twenties and be Life Science graduates with a relevant PhD. Familiarity with molecular biology techniques and post doctoral experience in the area of cell immortalisation will be advantageous.

The remuneration package will reflect your expertise and experience.

Interested candidates should write with full c.v. quoting reference RM/DJ, to:
The Personnel Department, ICI Pharmaceuticals, Mereside, Alderley Park, Nr Macclesfield, Cheshire SK10 4TG.

Closing date for applications is 29th March 1991. (5831)E

ICI *Pharmaceuticals*

INSTITUTE OF ORTHOPAEDICS
OSWESTRY, SHROPSHIRE

Post-doctoral Fellowship

Antisense Technology

The Imperial Cancer Research Fund is one of the largest charity research institutions in Britain with wide ranging interests in fundamental, applied and clinical research.

A post-doctoral position is available immediately in the ICRF Molecular Oncology Group at Hammersmith Hospital to work on the design and application of antisense DNA and RNA strategies against mutant ras oncogenes in human cancers. The project will involve manipulation of both modified oligodeoxynucleotides and novel retroviral vectors.

Candidates should have experience in molecular biology and recombinant DNA technology.

Further information is available from Dr Nick Lemoine on 081 740 3257.

The award will be tenable for up to three years with a salary of £16,286–£19,610 depending on age and experience.

(5842)E

Application should be made by sending full Curriculum Vitae and names and addresses of three referees to the Recruitment Office, Imperial Cancer Research Fund, PO Box 123, Lincoln's Inn Fields, London WC2A 3PX, quoting ref: 64/R.

SMOKING IS ACTIVELY DISCOURAGED

Imperial Cancer
Research
Fund

UNIVERSITY OF NEWCASTLE UPON TYNE

Department of Physiological Sciences

UNIVERSITY RESEARCH FELLOW

(POSTGRADUATE OR POSTDOCTORAL)

Applications are invited for a University Research Fellowship (post-doctoral) or Junior Research Fellowship (postgraduate) to collaborate on a project with Dr Adrian Rees. The Fellowship, which is financed by the University in order to foster and stimulate research projects of outstanding potential, is available immediately for a period of three years.

The project will investigate the neuronal circuits involved in the processing of auditory information in the mammalian midbrain. Electrophysiological recordings of neuronal responses to sounds will be used, in combination with the iontophoretic application of drugs, to study the excitatory and inhibitory processing mechanisms of neurones in the inferior colliculus.

Postdoctoral candidates should have a PhD in an area of neurophysiology, and experience with iontophoretic techniques would be an advantage. Previous experience in auditory physiology is *not* essential as appropriate training can be given.

Postgraduate candidates should have, or expect to gain, an upper second class honours degree or above in physiology, experimental psychology or a related subject. A good grounding in neurophysiology is desirable, and some knowledge of the special senses would be an advantage. Suitable candidates will be able to register for a PhD.

Salary will be at an appropriate point on the Grade 1B scale: £11,399-£13,495 for the Junior Research Fellowship or Grade 1A scale: £11,399-£18,165 pa, for the Research Fellowship according to qualifications and experience.

Further particulars may be obtained from the Director of Personnel, The University, 6 Kensington Terrace, Newcastle upon Tyne NE1 7RU, with whom applications (three copies), together with the names and addresses of three referees, should be lodged not later than 2nd April 1991. Postdoctoral candidates should also submit reprints of any publications (up to a maximum of 3). Informal enquiries may be made to Dr A Rees (tel: 091-222 6000 ext 6956, e-mail (JANET) : adrian.rees@uk.ac.newcastle, Department of Physiological Sciences, The Medical School, Newcastle upon Tyne NE2 4HH). (5822)E

UNIVERSITY OF
NEWCASTLE UPON TYNE

Research Studentships

Applications or nominations are invited for University Research Studentships in the following areas of research, selected for their promise of further development.

1 High resolution studies of the solid/liquid interface.
Dr P A Christensen, Department of Chemistry.
2 The development of an electron gun for investigating atoms and molecules by electron impact.
Dr T Reddish, Department of Physics
3 The role of viable but non-culturable forms in helicobacter pylori infection.
Dr M R Barer, Department of Microbiology.
4 The role of Ras and p53 gene mutations, human papillomavirus infection and chromosome rearrangements in skin cancer.
Dr J Rees, Department of Dermatology.
5 Acoustic phonetic analysis of dysarthria.
Dr G Docherty, Department of Speech.
6 Modulation of Ca^{2+} and voltage activated K^+ channels by protein phosphorylation.
Dr M A Gray, Department of Physiological Sciences.

Awards include a maintenance allowance having a value of £1000 per annum over and above that of a Research Council studentship. They include provision for the running costs of projects and access to travel funds.
Applicants should have, or expect to obtain, a first or upper second class honours degree. Full details of the studentships and application forms may be obtained from the supervisor named above in the relevant department at the University, Newcastle upon Tyne, NE1 7RU. Closing date 15th April, 1991.

(5832)F

JOHN INNES INSTITUTE

John Innes Centre for Plant Science Research, Norwich

RESEARCH IN PLANT AND MICROBIAL GENETICS, MOLECULAR BIOLOGY AND CELL BIOLOGY

PhD studentships starting in October 1991 will be available for many of the topics listed below. The grant for 1991/92 will be not less than £5,000 per annum.

1. Biochemical and molecular analysis of the *sickle* mutation of *Antirrhinum majus*, a chloroplast trait affecting leaf shape (C R Martin)
2. A genetic and molecular approach to flower development (E S Coen and R Carpenter)
3. Analysis of a chalcone synthase promoter from pea induced by heavy metals (P M Mullineaux and T H N Ellis)
4. Cellular and molecular analysis of early embryo development in peas using new chemically-induced mutants (T L Wang)
5. Developmental regulation in the morphologically complex prokaryote *Streptomyces coclicolor* (K F Chater)
6. Molecular signalling at the *Rhizobium*-legum interface in nitrogen fixation (N J Brewin)
7. Development of geminivirus-based genic delivey systems for cereals (P M Mullineaux: CASE with A Goldsborough, Plant Breeding International, Cambridge)
8. Polymerase chain reaction and direct DNA sequencing to identify microbes or to place them in phylogenetic context (J P W Young)
9. Genetic manipulation of plant viruses: modification of plant virus capsids to express foreign peptide sequences (G P Lomonossoff)
10. Molecular biology of spread and symptom induction of plant DNA viruses (J R Stanley)
11. Studies of the early events in the replication cycle of the pararetrovirus, cauliflower mosaic virus, including virion uncoating and differential regulation, assembly and structure of minichromosomes (S N Covey)
12. Cell and molecular biology of plant nucleolar proteins: role of insoluble nucleolar proteins and regulation of their assembly and disassembly during the cell cycle (P J Shaw and J Doonan)
13. Functional architecture of the plant cell wall investigated by biochemical, electron microscopic and monoclonal antibody studies (K Roberts)

For further details of any of these projects, and application procedure, contact Mrs Anne Williams, John Innes Institute, John Innes Centre, Colney Lane, Norwich NR4 7UH (Tel: 0603 52571; Fax: 0603 56844)

The John Innes Institute is associated with the AFRC Institute of Plant Science Research. (5826)F

DEPARTMENT OF BIOCHEMISTRY
University of Cambridge

PhD Research Projects for 1991

The Department currently has 25 members of academic staff, 52 senior research workers and 84 research students. At least 10 studentships should be available (plus earmarked* and CASE).
Examples of projects are:

Dr A Baker	Peroxisome biogenesis in higher plants
Dr D S Bendall*	Photosynthetic electron transport systems
Dr M D Brand	Control of energy flow in mitochondria and cells
Dr D M Carrington	The molecular biology of protozoan parasites
Dr H B F Dixon	Chemical enzymology
Dr D J Ellar	Novel biopesticides and immunotoxins from *Bacilli*
Dr P A Evans	Protein folding and design
Dr N J Gay	The biochemistry of *Drosophila* embryogenesis
Dr P J F Henderson	Molecular biology of membrane transport
Dr C J Howe	Molecular biology of photosynthetic organisms
Dr A P Jackson	Membrane recycling and coated vesicle regulation
Dr R J Jackson	Eukaryotic mRNA and viral RNA translation
Dr D Kerridge	The mode of action of antimycotic drugs
Sir Hans Kornberg	Molecular biology of carbohydrate transport
Dr E D Laue*	NMR studies and eukaryotic transcription factors
Dr P F Leadlay	Mechanisms of enzymes and antibiotic synthesis
Dr E N G Marsh	Enzymology of complicated chemical reactions
Dr B R Martin	Transmembrane signalling in eukaryotic cells
Dr J C Metcalfe	Control of normal eukaryotic cell growth
Prof R N Parham	Molecular biology of macromolecular assemblies
Dr P E Reynolds	Resistance to bacterial cell wall antibiotics
Dr P H Rubery	Molecular signalling in plants
Dr J O Thomas	Chromatin structure, and gene activation

*Earmarked studentships are available.

There are opportunities for graduate students to supplement income through Departmental teaching. Send enquiries and applications to: Dr P J F Henderson, Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW (0223 333622 or 333628). (5841)F

continued on page 1

COURSES

FUNDAMENTAL NEUROBIOLOGICAL APPROACHES TO DISEASE:

Brain Mechanisms in Neuropsychology

May 19-24, 1991

The Neurosciences Institute will sponsor an intensive, week-long course emphasizing the disease process as an object for biological research. The purpose is to introduce scientists to the basic physiology and pathology of selected human syndromes affecting behavior and to the opportunities they provide for solving fundamental biological problems.

The course program will review both clinical phenomena and pertinent areas of basic neuroscience in a format that includes lectures, clinical case presentations, and small group discussions. Instructors will include Ursula Bellugi, Hans-Joachim Freund, Joachim Fuster, Patricia Goldman-Rakic, Charles Marsden, Marek-Marsel Mesulam, Mortimer Mishkin, Fred Plum, Michael Posner, Marcus Reichle, Vilayanur S Ramachandran, Larry Squire, Thomas Thach, Larry Weiskrantz, Robert Wurtz, and Semir Zeki.

Applications are invited from scientists with advanced training and experience in neuroscience or related biological sciences. Participants' expenses will be reimbursed by the Institute.

For application forms or further information, please write to Dr W Einar Gall, Research Director, The neurosciences Institute, 1230 York Avenue, New York, New York 10021 or telephone (212) 570-8975.

(NW6515)D

continued on page 18

nature
the widest
international
selection of jobs in
science —
EVERY WEEK

FELLOWSHIPS/STUDENTSHIPS continued

POST DOCTORAL FELLOWSHIPS

The Biophysics Group (P-6) of Los Alamos National Laboratory has openings for post doctoral fellows in the following areas:

Vertebrate Photoreceptor Biochemistry and Function

Background: Ongoing studies focus upon biochemical, biophysical and computational analyses of the molecular ensembles that support photon transduction in the rod. Emphasis is given to G-protein function.

Current Program: The photoreceptor group in P-6 is now studying the mechanisms that govern the activation of rod phosphodiesterase with special emphasis on the role and function of the G-protein and its subunits. A new model for G-protein function has been developed, which emphasizes mechanisms for regulating rod sensitivity. We are also studying the coordinated regulation of the rod phosphodiesterase and guanylyl cyclase.

Red Blood Cell Biophysics and Biochemistry

Background: New findings have changed our approaches to the study of the determinant of red cell senescence in health and disease.

Current Program: The red blood cell section focuses upon the determinants of red cell aging. New findings help to explain time dependent changes in red cell membrane area and cytosolic volume. Computational analyses define the critical nature of the ratio of membrane area to cell volume. This relationship is helping to determine new approaches to developing improved red cell storage methods and understanding the molecular pathology of diabetic retinopathy.

Qualifications: Both positions require recent Ph.D. in Biochemistry, Protein Chemistry, Biophysics or related areas. Knowledge of enzymology, membrane and protein chemistry and excellent academic credentials are desirable.

Annual salary is $36,666 with excellent benefits.

Interested candidates should send a copy of their CV to Dr. M. W. Bitensky (P-6), MS K480, **PD-91-084,** Los Alamos National Laboratory, Los Alamos, New Mexico 87545. For additional information contact Dr. M. W. Bitensky at (505) 667-9420.

The Los Alamos National Laboratory is operated by the University of California and is an Affirmative Action/ Equal Opportunity Employer.

Los Alamos
NATIONAL LABORATORY

continued on page 19

CONFERENCES & COURSES

1991 CAMBRIDGE CONFERENCES: GENE THERAPY AND ANTISENSE THERAPEUTICS

San Diego, California

Pan Pacific Hotel

Wed. April 17

Non-ionic Oligonucleotide Analogues (Matagen™) As Anticodic Agents in Duplex And Triplex Formation
Dr. Paul O.P. Ts'o
The Johns Hopkins University

Oligonucleotide-Directed DNA Triple-Helix Formation: An Approach To Artificial Repressors?
Dr. L. James Maher, III
Cal Tech

Chimeric Antisense RNAs
Dr. Jonathan Izant
Yale University

Recent Progress In The Development Of Antisense Drugs
Dr. Chris Mirabelli
Isis Pharmaceuticals

POSTER SESSIONS

Gene Therapy
Dr. Theodore Friedmann
University of California San Diego

Towards Gene Therapy For Gaucher Disease
Dr. Donald Kohn
University of Southern California

Thur. April 18

Oligonucleotides: Cellular Uptake And Use As A Research Tool And Therapeutic Agent
Dr. Arthur M. Krieg
University of Iowa

Cutting The Chain Of Command: Specific Inhibitors Of Transcription
Dr. Jeff Holt
Vanderbilt University

Oligonucleotides: Informational Drugs
Dr. Jack S. Cohen
Georgetown Univ. Medical Center

Antisense: The Science And The Business
Dr. James Hawkins
SYNTHECELL Corporation
Ed: Antisense Res. & Dev.

Lymphocyte Gene Therapy
Dr. Ken W. Culver
NIH

HIV Infection and Gene Transfer Therapy
Dr. Douglas Jolly
Viagene, Inc.

Oncogenes As Transcription Factors
Dr. Inder Verma
The Salk Institute

Genetically Modified Cells: Applications For Intracerebral Grafting
Dr. Fred Gage
University of California San Diego

Roundtable Discussion: Gene Therapy-Public Perceptions, Ethics, And Regulation

Fri. April 19

Exploring The Use Of Anti-Sense, Enzymatic RNA Molecules (Ribozymes), As Therapeutic Agents
Dr. John Rossi
City Of Hope

Use Of Antisense c-*fos* RNA In The Analysis of C-*fos* In *sis* Transformed Cells And Human Osteosarcoma
Dr. Daniel Mercola
VA Hospital San Diego

Antisense Oligonucleotides Which Serve As A Substrate For RNase H
Dr. Joe Walder
University of Iowa & Integrated DNA Technologies

The Development Of Antisense Oligonucleotides As Therapeutics
Dr. Lyle Arnold
Genta

Roundtable Discussion: Future Research Directions In Antisense

Figure by The Gene Construction Kit
by Textco of W. Lebanon, NH

Synergy—gene therapy and antisense therapeutics-the unexpected combination that adds up to much more.

Boston, Massachusetts

Boston World Trade Center

Wed. April 24

Studying The Functions Of Maternal Genes In Xenopus Embryos With Modified Oligodeoxynucleotides
Dr. Douglas Melton/Tod Woolf
Harvard University

Chimeric Antisense RNAs
Dr. Jonathan Izant
Yale University

Oligonucleotides: Informational Drugs
Dr. Jack S. Cohen
Georgetown Univ. Medical Center

POSTER SESSIONS

Ribonuclease P: An Enzyme That Can Cleave Targeted mRNAs
Dr. Sid Altman
Yale University

Lymphocyte Gene Therapy
Dr. Ken W. Culver
NIH

Thur. April 25

Ribozymes As Potential Anti-HIV Therapeutics
Dr. Nava Sarver
NIH

Cutting The Chain Of Command: Specific Inhibitors Of Transcription
Dr. Jeff Holt
Vanderbilt University

Antisense Inhibition of *Myc* Family Gene Expression
Dr. Len N. Neckers
NCI

Antisense: The Science And The Business
Dr. James Hawkins
SYNTHECELL Corporation
Ed: Antisense Res. & Dev.

Correction Of Murine Mucopolysaccharidosis Type VII (MPS VII) By Bone Marrow Transplantation And Gene Transfer Therapy
Dr. Edward H. Birkenmeier
The Jackson Laboratory

The Unfolding Progress Of Human Gene Therapy
Dr. M. James Barrett
Genetic Therapies, Inc

Roundtable Discussion: Gene Therapy-Public Perceptions, Ethics, And Regulation

Fri. April 26

The Development Of Antisense Oligonucleotides As Therapeutics
Dr. Lyle Arnold
Genta

Antisense Oligonucleotides Which Serve As A Substrate For RNase H
Dr. Joe Walder
University of Iowa & Integrated DNA Technologies

Antisense Antivirals
Dr. John Goodchild
Worcester Foundation For Experimental Biology

Roundtable Discussion: Future Research Directions In Antisense

For Reservations Or Full Conference Program,
Please Call Or Fax:
International Conference Productions Corp.
550 West "B" Street, Suite 230
San Diego, California 92101
619/237-5010 • Fax 619/237-0358

Co-sponsored by BioWorld

CALL FOR POSTER ABSTRACTS

We Welcome Your Presentation

Just phone International Conference Productions Corporation at **(619) 237-5010** by March 27th, 1991 for poster abstract submission forms.

(NW6471)C

Södertörn Summer University and the Karolinska Institute Center for Structural Biochemistry
Summer School 1991

Understanding Protein Structure

NOVUM Research Park, Huddinge, Stockholm, August 18-23

The school will provide a broad overview of protein structure determination, analysis, and prediction. The talks will be at a level suitable for advanced PhD students and postdocs in molecular biology and structural biochemistry.

Topics

Protein Folding · Packing Principles and Architecture · X-ray Analysis · NMR Analysis · EM Analysis · Molecular Dynamics · Sequence Analysis · Structural Modelling.

Speakers

William Welch (USA), Ken Dill (USA), Carl-Ivar Brändén (Sweden), Jus Singh (UK), Greg Petsko (USA), Robert Kaptein (Netherlands), Sture Forsén (Sweden), Werner Kühlbrand (FRG), Herman Berendsen (Netherlands), Patrick Argos (FRG), Fred Cohen (USA), Arthur Lesk (UK), Michael Sternberg (UK).

Organizing committee

Gunnar von Heijne, Lennart Nilsson, Torleif Härd, Hans Hebert.

For information and application forms, please contact:
CONGREX, c/o Södertörn Summer University
PO Box 5619, S-114 86 Stockholm
Phone: Int+46-8-612 69 00. Fax: Int+46-8-612 62 92
Telex: 169 49 KRECON S

DEADLINE FOR APPLICATION IS May 15th, 1991

(W8310)D

EMBO PRACTICAL COURSE

MAKING ANTIBODIES IN BACTERIA AND ON PHAGE

September 28 — October 6, 1991

at IRBM Pomezia (Rome) — Italy

Organized by: Giovanni Cesareni; Riccardo Cortese; Giovanni Galfré and Gregory Winter

***Instructors:* from IRBM**
Riccardo Cortese, Franco Felici, Armin Lahm, Maurizio Sollazzo, Andreas Crameri, Giovanni Galfrè, Alessandra Luzzago, Anna Tramontano

— from the 2^D^ University of Rome — Tor Vergata
Luisa Castagnoli, Roberto Jappelli, Giovanni Cesareni

— from Cambridge Center for Protein Engineering and LMB
Gregory Winter

— from Cambridge Center for Protein Engineering
Michael Baier, Phillip Holliger, James Marks, Andrew Griffiths, Hennie Hoogenboom

— from LMB, Cambridge
Peter Jones

New technologies offer fresh ways of making antibodies and refining their properties — see Winter and Milstein "Man-made antibodies", Nature 349, 293—299 (1991).

The Course will focus on:
1. PCR cloning of antibody genes;
2. expression of soluble antibody fragments in bacteria;
3. assembly of repertoires of antibody genes from immunised animals and their expression on the surface of bacteriophage;
4. selection of phage and antibody genes by affinity chromatography.

There will also be lectures covering further aspects of antibody engineering and phage display technologies.

Vectors, primers and protocols will be distributed for the participants to take home.

The Course will be limited to 14 participants from European Research Institutions. Preferred applicants will be at the advanced predoctoral or the postdoctoral stage. Candidates must send a curriculum-vitae and a concise statement of the interest in this Course as related to their research project. Participation, including board and lodging, will be free of charge. No funds are available to cover travel expenses. **Send your application within June 15, 1991 to: Prof. Riccardo Cortese, IRBM, Via Pontina km. 30,600, 00040 Pomezia (RM), Italy. Fax 39—6—91093654** (W8311)D

continued on page 2

THE UNIVERSITY OF LEEDS
MOLECULAR ELECTRONICS

RESEARCH FELLOWSHIP
RESEARCH STUDENTSHIP

With a view to the development of molecular electronics we are investigating the design and synthesis of model peptides and their self-assembly behaviour in lipid bilayers to generate ion-conducting channels. The aim is to mimic the selective and controlled ion transport found in natural membrane proteins such as the voltage-gated sodium channel. This is a multidisciplinary project such as the voltage-gated sodium channel. This is a multidisciplinary project embracing the Departments of Biochemistry and Molecular Biology (Professor J B C Findlay and Dr P F Knowles) and the School of Chemistry (Dr N Boden). The present phase of the work focusses on establishing the fundamental principles and we now wish to expand this programme by recruiting.

I. **POSTDOCTORAL ORGANIC CHEMIST** who would take responsibility for the design and synthesis of novel peptides and lipids and participate in the structural characterization of the channels using NMR, X-ray diffraction and Fourier Transform IR.

Salary within the range £11,399-£13,495 on the 1A scale for Research Staff according to qualifications and relevant experience. Available for up to 3 years.

Application forms and further particulars may be obtained from and completed applications forwarded to the Registrar, The University of Leeds, Leeds LS2 9JT (tel 0532 333969 — direct line), quoting reference no 83/106.

(ii) **POSTGRADUATE RESEARCH STUDENT** (Physical Chemist or Physicist) who would similarly be involved in the structural studies and also in establishing the mechanism of ion transport. Maintenance according to SERC CASE terms with an industrial supplement.

Further details on the project will be sent on request. Applications including a curriculum vitae and the names and addresses of two academic referees should be sent to Dr P K Knowles, Department of Biochemistry and Molecular Biology, the University of Leeds, Leeds LS2 9JT.

Closing date for applications: 31 March 1991.

The University is an equal opportunities employer.

(5818)E

Elmore Medical Research Studentship: NOTICE

Applications are invited for the Frank Edward Elmore Medical Research Studentship holders of which are required to devote themselves to research in medicine or in some branch of the medical sciences according to a course proposed by themselves and approved by the Elmore Managers. The Managers would expect the work normally to be carried out in the University of Cambridge but in special circumstances part of the research may be carried out elsewhere under conditions approved by the Managers. The tenure will be for two years but may be extended for a second period not exceeding two years at the discretion of the Managers.

Under the terms of the trust women are not eligible for election to an Elmore Studentship.

The salary will be determined by the Managers within the range £10,699-£16,755 a year, according to the Student's age, qualifications and experience.

Application forms and further conditions of eligibility may be obtained from the Secretary of the Elmore Managers, Clinical School, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2SP and should be returned to him by 12 April 1991. (5833)F

---

nature
Still first with the best in science

COMMISSION OF THE
EUROPEAN COMMUNITIES

Directorate-General for Science, Research and Development

HUMAN GENOME ANALYSIS
(1990 TO 1992)

EC OPPORTUNITIES FOR TRAINING AND MOBILITY

- Bursaries & Subsidies -
- Grants -

SCOPE

Applications are invited for training/mobility grants provided for periods of up to 24 months from scientists who are citizens of a Member State of the European Communities.

INFORMATION

Detailed information can be obtained from the

Applicants' Guide to -
Bursaries & Subsidies -
- Grants -

at the following address:

Mr. A. Klepsch,
Commission of the European Communities,
DG XII/F6,
Human Genome Analysis Programme,
SDM 2/39,
rue de la Loi 200,
B-1049 Brussels.
Fax: + 32/2/235.53.65.

(W8307)H

AGRICULTURAL AND FOOD RESEARCH COUNCIL

STEM CELL MOLECULAR BIOLOGY

The AFRC announces a new coordinated research initiative aimed at understanding the molecular biology of stem cells in both plants and animals.

Stem cells are key components of plants and animals that, depending on type, have the capacity to differentiate into suites of specific cell types, tissues, organs or complete adults. For example stem cells of the haemopoetic system differentiate to generate all the cell types of the adult immune system. In the developing embryo of mammals there are stem cells at the 8 cell stage that can develop into complete adults, but by the 128 cell stage stem cell differentiation is restricted to the generation of major organs. Similarly plant meristematic tissues divide and differentiate, to form stems, roots, leaves and flowers. Unlike animal systems, however, the differentiated cells of plants can revert to meristematic form and develop into complete adult plants. Some microbial systems also undergo differentiation, for example some bacteria (eg the genus *Bacillus*) form highly dehydrated spores as a response to adverse conditions. These systems might prove to be useful models of the molecular processes by which differentiation is controlled.

The new initiative builds on existing strengths at AFRC institutes and Higher Education Institutions (HEIs) and will complement work being out by the Medical Research Council and the Science and Engineering Research Council. Funds will be allocated through open competition between AFRC institutes and HEIs. AFRC now seeks applications from the scientific community for basic research on the molecular biology of stem cells including the following topics:

- ★ embryonic stem cells
- ★ somatic stem cells
- ★ cell and molecular biology of totipotency
- ★ the use of stem cells in physiological, developmental and genetic studies in whole organisms.

Work on microbial systems and immunology, where relevant to the fundamental biology of stem cells or in which stem cells are used to probe the biology of plant or animal systems, will be encouraged. Grants will normally be for three years in the first instance, commencing 1 January 1992, but with the possibility for renewal.

Application forms and further details can be obtained, until 13/14 April 1991, from the AFRC Higher Education Branch, Wiltshire Court, Farnsby Street, Swindon SN1 5AT (Telephone: 0793 514242 ext 219) and subsequently from the AFRC Higher Education Branch, Polaris House, North Star Avenue, Swindon SN2 1UH (Telephone 0793 413200). Applications should be submitted by 15 July 1991. (5812)H

FELLOWSHIPS/STUDENTSHIPS continued

THE ROYAL SOCIETY

EUROPEAN SCIENCE EXCHANGE PROGRAMME

POSTDOCTORAL FELLOWSHIPS

The Royal Society invites applications from UK residents for fellowships tenable in laboratories of applicants' choice in:- Austria, Belgium, Denmark, Finland, France, Germany, Greece, Hungary, Republic of Ireland, Italy, Netherlands, Norway, Poland, Portugal, Spain, Sweden, Switzerland and Turkey. Residents in these countries should apply to the Royal Society's partner organization in the country (generally the research council or academy). The number of fellowships that are available for each country varies depending upon the extent of funding by the Society's partner in that country.

Fellowships are normally for a full academic year, although applications for less than a full academic year, but not less than six months, will also be considered, as will applications for up to two years.

Preference is given to newly or recently postdoctoral applicants and the award is based on UK university lecturer rates, taking account of cost of living differences. Candidates holding permanent appointments in the UK are also eligible to apply; in such instances the award will take into account any continuing salary which the candidate will receive.

The closing dates for applications are 10 May and 12 January, for awards to be taken up approximately 8 months after these dates.

Further details and application forms can be obtained from: **The Executive Secretary (ref: ESEP/N), The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG. Telephone: 071-839 5561 (ext. 218).** (5817)E

continued on page 21

ANNOUNCEMENTS

The European Commission has contracted EURO-TOP CONSULTANTS to promote and organize the presence of European companies and laboratories dealing with biotechnology at FENABIO-BIOLATINA 91 an exhibition and fair which will be held in Sao Paulo, Brazil on July 6-10 1991.

Contact: Mr SCHAUS Marc, EURO-TOP, Residence Floride,
490 Avenue Louise Bte9, B.1050 Brussels
BELGIUM
Tel: (32.2) 649.59.94/646.37.03
Fax: (32.2) 640.37.59 (W8298)G

CONGRESS

2ND INTERNATIONAL CONGRESS ON AMINO ACIDS AND ANALOGUES

Vienna, August 5-9th 1991

Chemistry–Medicine–Biology–Nutrition–Technology
Correspondence: Gert Lubec, University of Vienna, Dpt of Paediatrics,
Währinger Gürtel 18, A 1090 Vienna, Austria
Tel: (222) 23 40 424 or (222) 40 400 3166 Telefax (222) 40 400 3238

TOPICS: *analysis, separation, synthesis, biosynthesis, nutrition, parental nutrition, cross linking amino acids, updating of tryptophan, amino acids and the immune system (incl AIDS), amino acids in sports medicine, racemixation, modification of amino acids (eg methylation), new roles for arginine, neurochemistry incl excitatory amino acids, food chemistry and technology (includ radiated food amino acids), polyamines, renal physiology and amino acids, amino acids in nephrology in gastroenterology, amino acids and behaviour, inborn errors of metabolism, molecular biology of amino acids, therapeutic implications, pharmacology, amino acids as free oxygen radical scavengers, stable isotopes etc.*

CALL FOR PAPERS!!!
PLEASE ORDER THE PRELIMINARY PROGRAM!! (8285)Q

STUDENTSHIPS continued

The Royal Free Hospital

University of London

Research Studentship in Vascular Cell Biology

Applications are invited from current or prospective graduates in Molecular or Biological Sciences wishing to study for a PhD. The studentship is available from October 1991 and the successful candidate will have access to the resources of Glaxo Group Research who are funding the project.

The project aims to isolate, culture and characterise microvascular endothelium derived from patients with Inflammatory Bowel Disease to further our understanding of the mechanism of microvascular injury and its role in this group of diseases. Both cell and molecular biology techniques will be required to evaluate the role of cytokines and adhesion molecules.

The student will benefit from both a basic research, though clinically relevant, environment with the Inflammatory Bowel Disease Study Group at the Royal Free Hospital whilst maintaining interaction with a major industrial organisation.

Applicants should send a cv to:
Dr. A. Wakefield, The Academic Department of Medicine, Royal Free Hospital School of Medicine, Pond Street, London NW3 2QG (5844)F

Glaxo Group Research

CENTRE FOR REPRODUCTIVE BIOLOGY, EDINBURGH

STUDENTSHIPS 1991

Research Projects

The studentships are awarded on a competitive basis and supported by the MRC, SERC, Edinburgh University, Wellcome Trust and other industrial sponsors.

1. **Cell biology of oxygen radical generation of human sperm**
2. **Melatonin and the biological clock in mammals**
3. **Molecular basis of inadequate corpus luteum formation**
4. **Role of immune system in the physiology of labour**
5. **Physiology and pharmacology of the cervix in pregnancy**
6. **Development of variant genotypes in mouse chimaeras**
7. **Regulation of steroid secretion by the corpus luteum**

Applications, including curriculum vitae and the names of two referees, to Dr T A Bramley, Postgraduate Studies Committee, Centre for Reproductive Biology, 37 Chalmers Street, Edinburgh EH3 9EW.

Closing date: 3rd April, 1991. (5836)F

continued on page 22

nature
the widest international selection
of jobs in science
– EVERY WEEK

CONFERENCES/WORKSHOPS

Fundación Juan March

International Meetings on Biology

INNOVATIONS ON PROTEASES AND THEIR INHIBITORS: FUNDAMENTAL AND APPLIED ASPECTS

30 September-3 October, 1991. Madrid (Spain)

Program: The workshop will cover different aspects of proteases and inhibitors, such as expression, molecular genetics and engineering, evolution, biophysics, 3D-structure/function, and biotechnological and biomedical applications. **Format:** The meeting will take place in the facilities of the Fundación Juan March. There will be long oral presentations, followed by discussions, as well as ample time for informal exchanges. Participants are encouraged to present their own work in the form of posters. **Speakers:** F.X. Avilés, A.J. Barrett, T. Blundell, W. Bode, P. Carbonero, R.W. Carrell, T.E. Creighton, C.S. Craik, E.W. Davie, L.D. Fricker, H. Fritz, R. Huber, A.J. Kenny, H. Neurath, A. Puigserver, C.A. Ryan, J.J. Sánchez-Serrano, R.L. Stevens, K. Suzuki, V. Turk, J. Vendrell, K. Wüthrich. **Organizer:** Dr. Francesc X. Avilés, Departamento de Bioquímica e Instituto de Biología Fundamental. Universidad Autónoma de Barcelona, Bellaterra, Barcelona (Spain). **Participants:** A maximum of 25. A number of places could be assigned to young scientists. No registration fee required. A limited number of fellowships will be available to help cover travel and accommodation expenses. Desire to obtain a fellowship should be expressed in the application letter. **Applications:** Should be sent to Fundación Juan March, Castelló 77, 28006 Madrid (Spain). Tel.: 34-1-435 42 40, Fax: 34-1-576 34 20 and should consist of an abbreviated curriculum vitae, a short abstract of the possible work to be presented as a poster, and a brief statement of the reasons to participate. No application forms will be issued.
Deadline: May 31st, 1991. (W8293)V

PRELIMINARY ANNOUNCEMENT

8th International Conference on Second Messengers & Phosphoproteins

(The Biology & Medicine of Signal Transduction)

GLASGOW, SCOTLAND, 3–8th AUGUST 1992

This multidisciplinary conference will encompass current research on cellular signal transduction and will therefore be of interest to biomedical scientists in various disciplines including pharmacology, endocrinology, cell biology, biochemistry, physiology, neuroscience and clinical medicine. This triennial Conference will comprise Plenary Lectures, Symposia and Workshops, and will include contributions from invited speakers and presentations of submitted papers.

Further information and future announcements can be obtained from **Dr. Pauline R. M. Dobson, General Secretary, 8th International Conference on Second Messengers & Phosphoproteins, Institute of Endocrinology (HMCB), University of Sheffield Medical School, PO Box 596, Beech Hill Road, Sheffield S10 2RX, U.K. (Fax 44 + (0)742 739976).** (5803)C

COURSES

EMBO PRACTICAL COURSE ON

XENOPUS EMBRYOLOGY

Oxford, UK, Sept. 8-15, 1991.

Organizers: J.M.W. Slack and J.C. Smith.

Enquiries to Dr J. Slack, ICRF Unit, Dept. Zoology, South Parks Road, Oxford OX1 3PS, UK. Tel: 0865 59977.

Closing date for applications: April 30, 1991.

(5813)D

FELLOWSHIPS/STUDENTSHIPS continued

UNIVERSITY OF GLASGOW
School of Biological Sciences
DEPARTMENT OF BOTANY

Applications are invited for an SERC-funded earmarked studentship, tenable from 1 October 1991. This earmarked studentship is to study the structure of bacterial light-harvesting complexes and the successful candidate will join a multidisciplinary group involved in structural studies on membrane proteins. Candidates should possess or expect to obtain a First or Upper Second Class Honours Degree in Botany, Biochemistry, Molecular Biology or a related subject. Applications, including a *curriculum vitae*, and the names and addresses of two referees, should be sent to **Professor R J Cogdell, Department of Botany, University of Glasgow, Glasgow G12 8QQ.** (5819)F

SIDNEY SUSSEX COLLEGE, CAMBRIDGE
Stanley Elmore Senior Research Fellowship in the Medical Sciences

The College Council invites applications from men and women for the above Fellowship, for the furtherance of medical research. The Fellowship will commence not later than 1 October 1991 and is tenable for three years in the first instance but may be renewed for a further period which normally would not exceed three years. Stipend and allowances up to £16,755 per annum, with membership of the Governing Body and the usual Fellowship rights.

Particulars may be obtained from the Master, Sidney Sussex College, Cambridge CB2 3HU. Applications should reach the Master NOT LATER THAN 30 APRIL 1991. (5837)E

ST MARY'S HOSPITAL MEDICAL SCHOOL
(a constituent College of Imperial College of Science, Technology & Medicine)
(University of London) Norfolk Place, London W2 1PG
DEPARTMENT OF BIOCHEMISTRY & MOLECULAR GENETICS

RESEARCH FELLOWSHIP — DEVELOPMENTAL GENETICS

An MRC funded position is available to work on isolation of genes from chromosome 22q11 involved in a human developmental field defect, DiGeorge syndrome, which is thought to involve a disruption of the contribution of the neural crest to organogenesis. Ideally, the candidate would have experience in molecular genetics and/or embryology. The post is available immediately for three years, although appointment could be deferred for the right candidate. The position would suit an experienced research assistant or a scientist looking for a first post-doctoral position. For further details telephone Dr Peter Scambler on 071 723 1252 ext 5382.

Applications in the form of a full CV with the names of 2 referees should be sent to the Personnel Department at the above address. Please quote Ref: RF/PS. Closing date: 3rd April, 1991. (5810)E

UNIVERSITY OF ABERDEEN
Department of Geology and Petroleum Geology
OCCIDENTAL POST-DOCTORAL RESEARCH FELLOW IN PETROLEUM GEOLOGY

Applications are invited for the Occidental Fellowship in Petroleum Geology. The appointee will work in collaboration with DR N H Trewin of The University and the geological team at Occidental Petroleum (Caledonia) Ltd in Aberdeen. The research project is a study of the sedimentology and diagenetic history of early Cretaceous reservoir sandstones of the Scapa Field area, North Sea. Postgraduate experience in sedimentary petrology, sedimentology, diagenesis and log interpretation is preferred. Publication of results is expected. Initial appointment will be for two years on a salary scale commencing at £11,399.

Applications by letter and CV (two copies) to the Personnel Department, University of Aberdeen, Regent Walk, Aberdeen AB9 1FX by 30th April 1991 quoting reference number LG/073. (5828)E

COURSES continued

HEART RESEARCH

The Coronary Artery Disease Research Association supports investigations into the non-invasive, presymptomatic diagnosis of cardiovascular disease, a fuller understanding of atherogenesis and the evaluation of measures to arrest/protect against the disease process with the aim of preventing premature death and disability.

The charity has a continuing interest in the cardiological applications of magnetic resonance techniques with particular emphasis upon ischaemic heart disease.

Applications for the support of research consonant with the charity's aims are now invited. Application forms are available from the address below and should be completed for return **no later than 11th May, 1991.**

Tavistock House North
Tavistock Square
London, WC1 9TH
Telephone: 071-387 9779

CORDA
the heart charity

(5807)D

METHODS IN COMPUTATIONAL NEUROSCIENCE,
August 4–31, 1991.

This course is for 20 advanced graduate students, postdoctoral fellows and faculty in neurobiology, physics, electrical engineering, computer science, mathematics, or psychology with an interest in "Computational Neuroscience". A background in programming (preferably in C and UNIX) is highly desirable.

Directors: James M. Bower, Christof Koch, Associate Director: Kenneth D. Miller, Computation and Neural System Program, California Insitute of Technology. Partial financial aid is available to qualified applicants. Application Deadline: May 15, 1991. For further information and applications, contact, **Admissions Coordinator, Marine Biological Laboratory, Woods Hole, MA 02543, USA, (508) 548–3705, ext. 216.** (NW6502)D

---

nature
— the world's most
prestigious
weekly journal
of science

3rd EMBO Practical Course on ADVANCED DIGITAL LIGHT MICROSCOPY
6-17 October 1991, EMBL, Heidelberg, FRG

Organizers: Ernst H.K. Steizer & Jan De Mey

The course is intended for students who have a professional interest in modern light microscopic techniques, such as low light level video fluorescence microscopy, video differential interference contrast microscopy, confocal fluorescence or confocal reflection microscopy and related techniques. The ideal student needs to learn these techniques to follow his/her scientific interests and he/she has access to, or intends to acquire appropriate equipment. The ideal candidate is at the pre- or post-doctoral level and will immediately use the techniques and information taught in the course.

Speakers from the USA and Europe will introduce the students to:

— principles of transmission and fluorescence light microscopy
— Fluorescence recovery after photo bleaching
— Fluorescence activation
— Cameras for microscopy
— Electronic equipment
— Image processing soft- and hardware
— Confocal microscopy
— Optical trapping/optical tweezers
— Ratio imagaing
— Gold imaging
— Chromophores
— Sample preparation

Sixteen students from the EMBO member states will participate. The deadline for applications is 24 June 1991. The application should include a curriculum vitae, a short description of the current project and the reason for attending this course. Please send the complete application to: Ingeborg Fatscher, EMBL, Meyerhofstr 1, 6900 Heidelberg, FRG (Tel: 0049-6221-387430, Fax: 0049-6221-387306). (W8295)D

Typesetting: Typedesign Ltd, London EC2 and J. W. Arrowsmith Ltd, Bristol. Printing: Chase Web Offset, Plymouth, a St Ives Company, and The Riverside Press Ltd, Whitstable, Kent. William Byrd Press Inc., Richmond, Virginia, USA and Obun Printing Co., Bunkyo-ku, Tokyo, Japan. Published by MACMILLAN MAGAZINES LTD, 4 Little Essex Street, London WC2R 3LF—14 March 1991.

Animal Experimentation

The Consensus Changes

Edited by Gill Langley

Animal experimentation remains a highly controversial issue. Proponents and opponents alike resort to a variety of scientific and moral arguments, but these are rarely presented in other than a fragmentary way.

Animal Experimentation: The Consensus Changes takes a wide-ranging look at questions of practice and philosophy, policy and politics.

Do we really consider ourselves to be part of the animal kingdom? To what extent do other animals feel pain? How reliable are laboratory animal models in medical research and toxicology? How are the scientific and legislative communities responding to public concern about animal experiments?

This collection of 10 essays by experts in disparate fields – medical research, anti-vivisection, science policy, legislation, humane research and philosophy – provides a stimulating and thought-provoking overview of current thinking on animal experimentation.

> *"The result of this multi-authored, multi-opinioned book is not, as might be supposed, an undisciplined cacophony. On the contrary, the book has a curious harmony. Running through each chapter is a concern for the humane treatment of animals, and the different ways this is expressed actually give the book a coherence that a more one-sided book would lack. The fact that people of very different views can contribute to the same volume lends weight to the idea that the consensus on the scientific use of animals might be changing. Perhaps it really is possible for people with differing views on this difficult subject to learn from each other. Certainly, this book will help them to do so."*
>
> **New Scientist**

> *"This book should be compulsory reading for all those engaged in animal research"*
>
> **Nature**

Contents

1. **Are you an animal?** *Mary Midgley*
2. **Ill-gotten gains?** *Tom Regan*
3. **Evidence for pain and suffering in other animals** *Margaret Rose and David Adams*
4. **Methods and practices of animal experimentation** *Erik Millstone*
5. **Animal experiments—a failed technology** *Robert Sharpe*
6. **Trivial and questionable research on animals** *Clive Hollands*
7. **Replacing animal experiments** *Martin Stephens*
8. **The scientist's responsibility for refinement: a guide to better animal welfare and better science** *David B. Morton*
9. **Plea for a sensitive science** *Gill Langley*
10. **Legislation and the changing consensus** *Judith Hampson*

Appendix: Current Legislation Initiatives in the USA *Holly Hazard*

Index

1990 280pp 234 x 156mm Hardback £27.50 0 333 45382 4 Paperback £10.95 0 333 45383 2

ORDER FORM

Order from your usual bookseller, or return this form to: Dionne Stocking, Macmillan Press Ltd, Houndmills, Basingstoke, RG21 2XS, UK

Please send me:

______ copy/ies of **Animal Experimentation** @ £27.50 hardback 0 333 45382 4

______ copy/ies of **Animal Experimentation** @ £10.95 paperback 0 333 45383 2

☐ I enclose a cheque for £ ____________ made payable to Macmillan Press Ltd *

☐ Please charge my Visa/Access/Mastercard/Amex/Diners Account No. ____________

Account Name ____________

Account Address ☐ (if different to delivery address below) ____________

Signature ____________ ____________

Expiry Date ____________ ____________

Name ____________

Adddress ____________

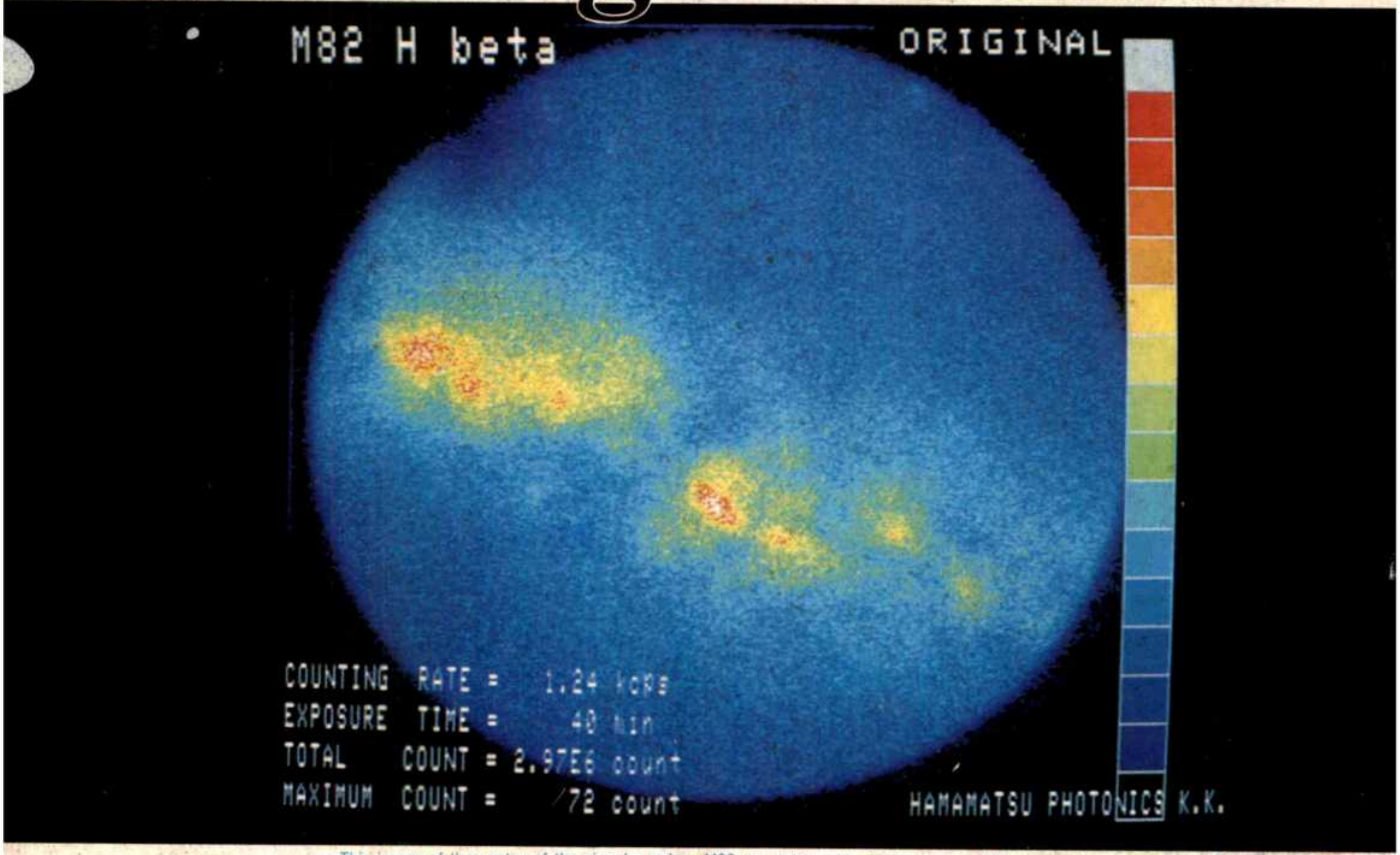
____

____________ Postal/Zip Code ____________ Country ____________

* Postage and packing free if payment sent with order.

NAT

A Single Photon



This image of the center of the singular galaxy M82 was taken with the Hamamatsu Photon-counting Image Acquisition System, PIAS. (Photo courtesy of the National Astronomical Laboratory; Kiso Laboratory of the University of Tokyo)

In the study of the behavior of photons we seek to comprehend the true nature of light. Photons behave like waves, but also like particles. Is it possible to define the nature of a single photon? What could such an achievement mean for science and humanity?

Research in photonics has yielded more than just paragraphs for textbooks. Our company's attempts to define the photon have brought unprecedented sensitivity to Hamamatsu photodetectors, which now have a wide range of practical applications. But this is only one example. Photonics offers a different viewpoint for unsolved problems in other fields of science as well.

At our Central Research Laboratory in Japan, research teams are studying how the findings from our investigations in photonics apply to molecular science, medical research communication, and many other fields.

As a world leader in light research, Hamamatsu Photonics not only strives to produce the most precise devices for light related research, but also to proceed on its own with basic research into the contradictions of the current understanding regarding the nature of light. We believe that working on the unsolved problems of photonics may lead to practical tools for a better life for all humanity and the achievement of world peace.

Teruo Hiruma
President, Hamamatsu Photonics K.K.

The photon is our business.

HAMAMATSU PHOTONICS K.K.

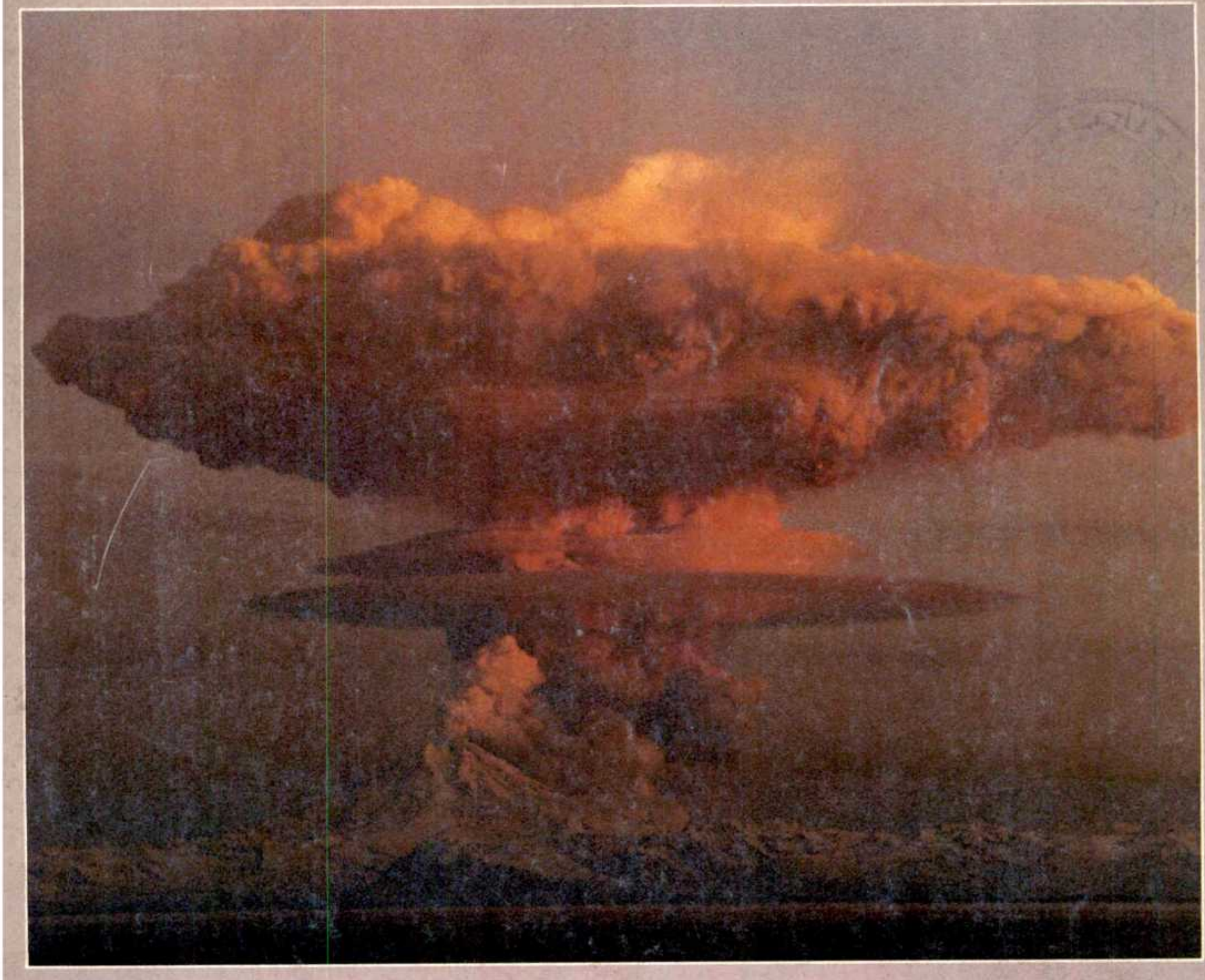
325–6, Sunayama-cho, Hamamatsu City, 430 Japan
Telephone: 053/452–2141, Fax: 053/456–7889
Telex: 4225–186

Reader Service No. 619

nature

INTERNATIONAL WEEKLY JOURNAL OF SCIENCE

Volume 350 No. 6320 25 April 1991 £2.50

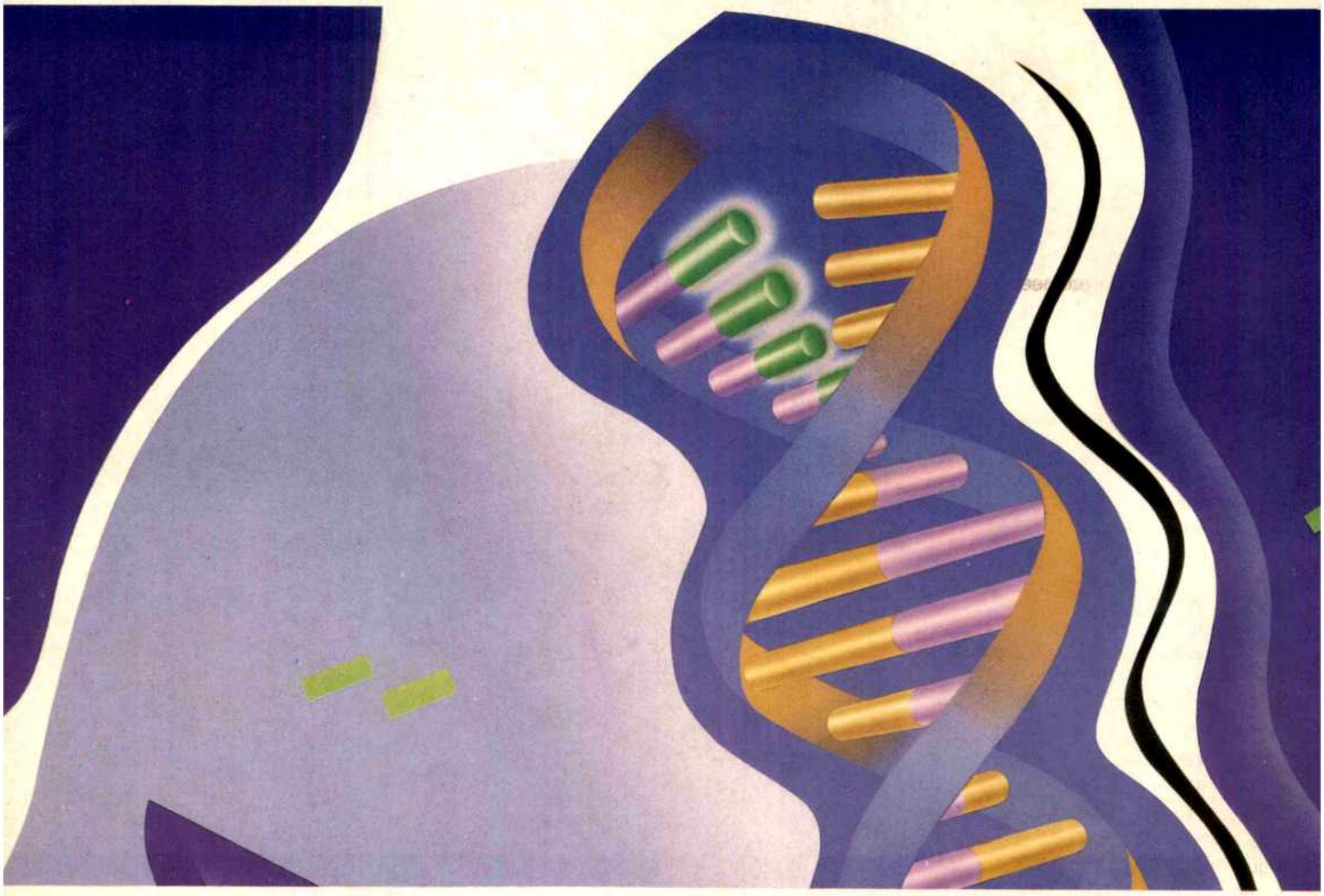


PREDICTING VOLCANIC ERUPTIONS

High-affinity nerve growth factor

The ring around supernova 1987A

GENE & RANDOM PRIMED IMAGES™ NON-ISOTOPIC DETECTION KITS



The most convenient, most complete nucleic acid detection system based on chemiluminescence for Northern/Southern blots.

Gene Images™ Non-Isotopic Nucleic Acid Detection Kit yields rapid, hard copy results of DNA/RNA analysis safely, simply and accurately.

- High sensitivity–detects sub-picogram quantities of nucleic acid.
- Qualified high-sensitivity nylon membranes yielding minimal background.
- High specific activity streptavidin-alkaline phosphatase conjugate and easy-to-use blocking solution included.
- Lumi-Phos™ 530* detection reagent supplied in a convenient, spray applicator bottle.
- Blots may be re-probed and re-exposed.

Gene Images™ is supported by Random Primed Images™ Biotin Labeling Kit for rapid labeling of DNA probes with biotinylated nucleotide (Biotin-21-dUTP).

- Utilizes exonuclease-free Klenow.
- Resulting probes are stable for up to 1 year at -20° C.
- Contains sufficient reagent for 50 labeling reactions.
- Probes are suitable for all types of hybridization.

To order Gene Images™ (Product No. 75300) and Random Primed Images™ (Product No. 75200), contact United States Biochemical Corporation, P.O. Box 22400, Cleveland, OH 44122. 800/321-9322 Fax: 800/535-0898. International: 216/765-5000 Telex: 980718 Fax: 216/464-5075.

USB™
United States Biochemical

Reader Service No.590

USB INTERNATIONAL OFFICES: Cambridge, England • Bad Homburg, Germany INTERNATIONAL DISTRIBUTORS: BioTrade—Austria • Cambridge BioScience—Cambridge, England • Getter (2000) Ltd.—Tel-Aviv, Israel • Integro B.V.—Holland • Kebo—Spanga, Sweden • Ballerup, Denmark • Espoo, Finland • Oslo, Norway • Lucerna-Chem AG—Lucerne, Switzerland • Quimigranel-SA — Spain • Societa Italiana Chimici—Roma, Italy • Sopar-Biochem SA—Belux • Touzart et Matignon—Paris, France • Toyobo Company, Ltd.—Osaka, Japan • Trace Scientific—Baulkham Hills, Australia

Lumi-Phos™ 530 is a trademark of Lumigen, Inc. U.S. Patent # 4,857,652

Pure nucleic acids – QIAGEN does them all !

IAGEN is all you need

o matter what type of nucleic acid
ou want to isolate –
IAGEN columns are all you need.

t´s this fast

. **Adsorb** a cell lysate
. **Wash** away contaminants
. **Elute** pure nucleic acid

t´s this convenient

eady-to-use kits for
ll types of nucleic acids –
Column sizes for any
ize prep –
ravity flow alone results
optimal separations –
umerous lab-tested protocols
re included in our booklet
HE QIAGENOLOGIST.

t´s this pure

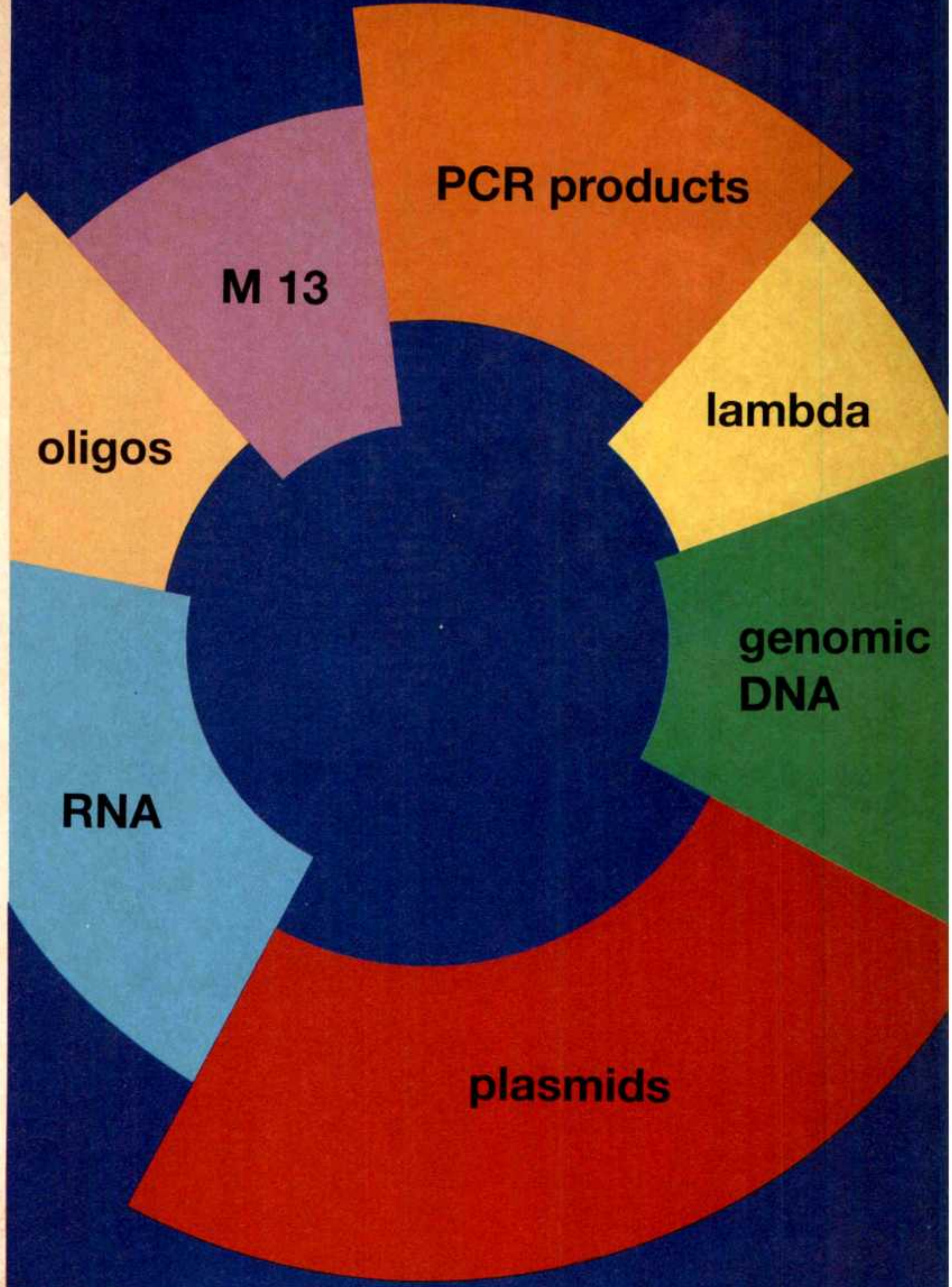
IAGEN-purified nucleic acids are
qual in purity to those isolated on
sCl gradients.
ubsequent applications such as
ransfections, clonings, sequencing,
ranscription and translation
eactions proceed with optimal
fficiency.

t´s this versatile

t the heart of the system is a
nique anion exchange resin with
e broadest possible separation
ange – QIAGEN.
enables the selective isolation of
e various classes of nucleic acids,
cluding dsDNA, ssDNA and RNA.

or more information

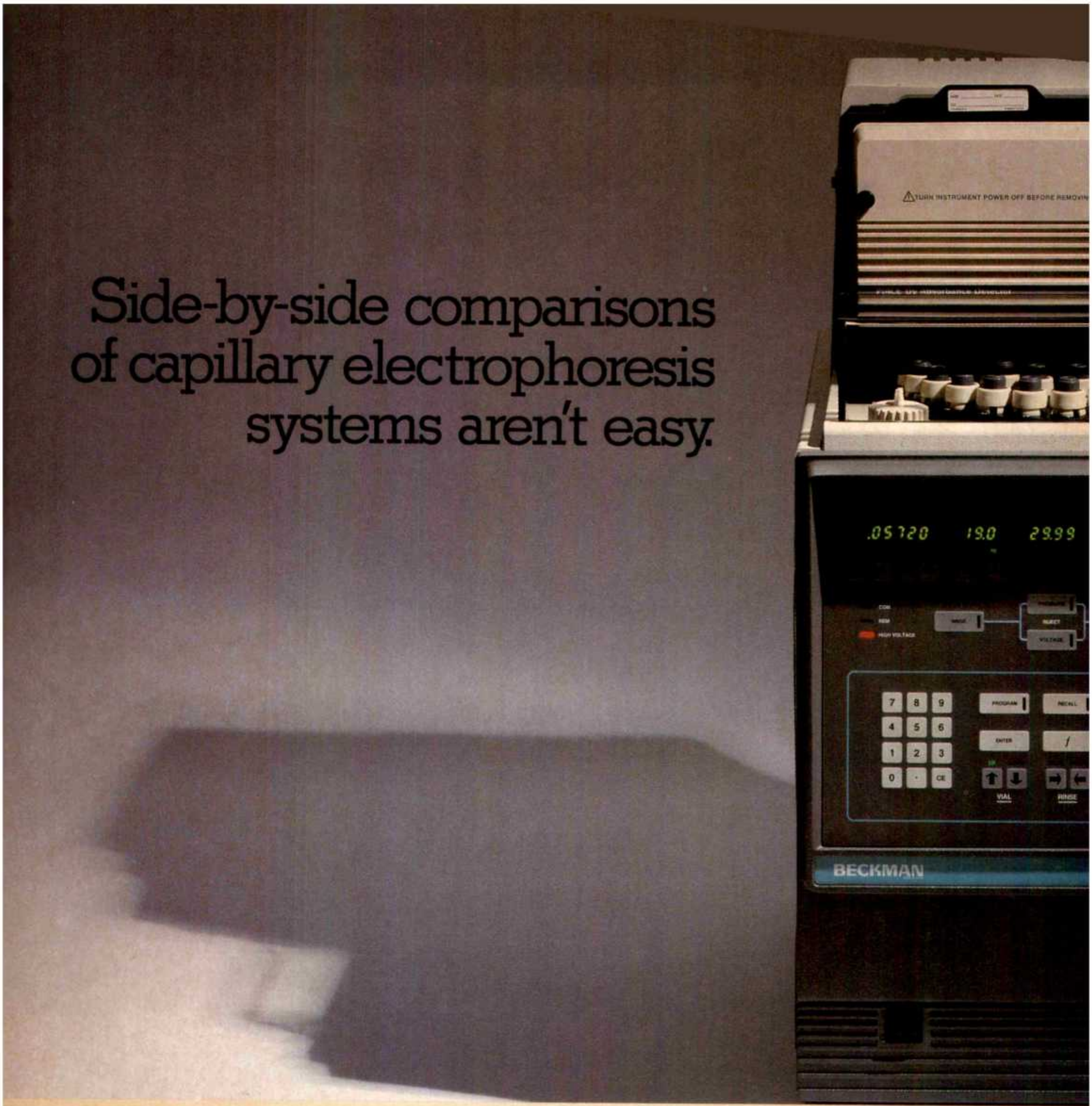
all or write to QIAGEN Inc.,
IAGEN GmbH or your nearest
istributor.



Reader Service No.467

USA/CANADA: QIAGEN Inc., Chatsworth, CA 91311, Phone (800) 426-8157, (818) 718-9870, Fax (818) 718-2056
GERMANY: DIAGEN GmbH, Niederheider Str. 3, D-4000 Düsseldorf 13, Phone (0211) 79 30 37, Fax (0211) 79 04 44
DISTRIBUTORS: AUSTRALIA: Phoenix Stansens Scient.Div. (3) 544 8022 **AUSTRIA:** Bio-Trade (222) 828 46 94
BENELUX: Westburg B.V. (NL-33) 95 00 94 **FRANCE:** Coger (1) 45 33 67 17 **ISRAEL:** Bio-Lab Laboratories Ltd.
(2) 52 44 47 **ITALY:** Genenco (M-Medical) (55) 67 64 41 **JAPAN:** Funakoshi Co., Ltd. (3) 5684 1622
KOREA: LRS 924-8697 **PORTUGAL:** Izasa Portugal, S.A. 758 07 40 **SCANDINAVIA:** Kebo Lab: Denmark: (44) 68 18 00,
Finland: (90) 437 56 40, Norway: (02) 30 11 20, Sweden: (08) 621 34 00 **SPAIN:** Izasa S.A. (3) 401 01 01
SWITZERLAND: Kontron Instruments AG (1) 733 5 733 **TAIWAN:** Formo (2) 736 7125 **UK:** Hybaid Ltd. (81) 977 3266

QIAGEN


Side-by-side comparisons of capillary electrophoresis systems aren't easy.
.05720 19.0 29.99
BECKMAN

Because nothing compares with the total automation of the P/ACE™ System.

Truly unattended methods development...

Powerful PC control allows you to vary parameters within methods. And link methods together. Automatically change rinse sequences. And inlet and outlet buffers. Samples and sample volumes. Temperature. Voltage. Detection wavelength.

And much more. In fact, with P/ACE, you automatically increase your productivity.

And automated fraction collection...

P/ACE is the *only* system available today with the capability to collect peaks after separation for further purity checks and identification. Automatically.

Proven worldwide.

The growing network of P/ACE users spans the globe. Like no other CE system, P/ACE is improving results and increasing productivity in leading laboratories every day.

From Beckman — the leader in capillary electrophoresis.

Though we make everything else so easy, we're proud to make side-by-side comparisons difficult.

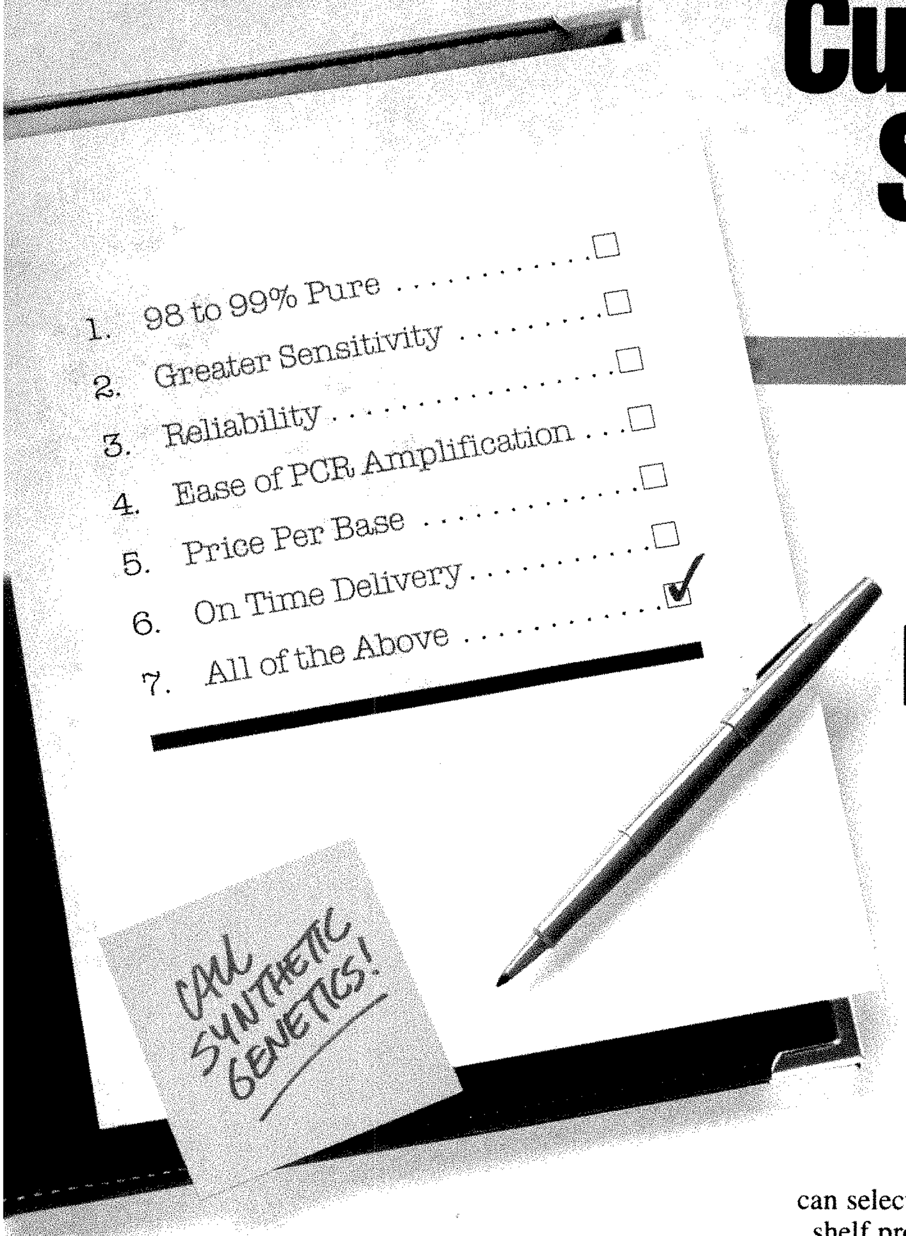
For more information, call 800/742-2345 (in the U.S.), or write to Beckman Instruments, Inc., 1050 Page Mill Road, Palo Alto, CA 94303-0803. Offices in major cities worldwide.

BECKMAN

Reader Service No. 691

©Copyright 1990, Beckman Instruments, Inc.

When It Comes To Custom DNA Synthesis...



1. 98 to 99% Pure ....☐
2. Greater Sensitivity ....☐
3. Reliability ....☐
4. Ease of PCR Amplification ...☐
5. Price Per Base ....☐
6. On Time Delivery ....☐
7. All of the Above ....☑

CALL SYNTHETIC GENETICS!

What Really Matters Most?

All of the above and more is what you can expect with your custom DNA from Synthetic Genetics.

Synthetic Genetics offers Gel Pure Custom Services along with the standard HPLC so that you are certain of the highest quality DNA for your sophisticated research projects.

Whatever your research requirements, we can deliver virtually any DNA type or you can select from one of the largest libraries of off-the-shelf probes and primers available anywhere. These probes and primers offer high sensitivity and a greater affinity for PCR amplification.

When your research requires chemically modified synthetic or normal DNA, look to the company with all of the above...Synthetic Genetics.

synthetic genetics

The Leader in DNA and Antisense Technology

1-800-562-5544 or 1-619-793-2661

See us at FASEB, Booth #2618

Reader Service No. 680

3347 Industrial Court, San Diego, CA 92121 • Fax (619) 793-2666

ECL

Light-based detection for Western blots

Trying to choose between ^{125}I, colorimetric and other non-radioactive systems?

With the new ECL Western blotting system, which complements our other systems, we can now help you choose the technology, radioactive or non-radioactive, to best suit your experimental needs.

The ECL (enhanced chemiluminescence) system is based on using horseradish peroxidase labelled second antibodies, or biotin-streptavidin systems.

In the presence of the ECL Western blotting detection system an enhanced light signal is created.

- 10 times as sensitive as colorimetric systems
- Results in minutes, often seconds
- Economical use of primary and secondary antibodies
- Simple protocols
- Carry out normal blotting and immunodetection techniques and then add ECL detection system for 1 minute
- No fading of results; hard copy results on film

ECL Western blotting detection system RPN 2106
(sufficient for 4000cm^2 of blots)

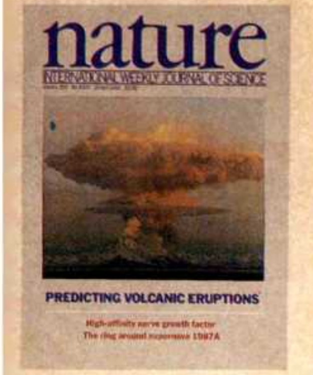
Contact your local Amersham office for further details

Amersham International plc
Amersham UK

Amersham
Bringing science to life

UK Sales Aylesbury (0296) 395222 **Amersham Australia Pty Ltd** Sydney (02) 888-2288 **Amersham Belgium SA/NV** Brussels (02) 770 0075
Amersham Buchler GmbH & Co KG Braunschweig West Germany (0 53 07) 2 06-0 **Amersham Canada Ltd** Oakville ONT (416) 847-1166
Amersham Corporation Arlington Heights, IL USA (800) 323-9750 **Amersham Denmark ApS** Birkerød 45-82 02 22 **Amersham France SA** Les Ulis (1) 69 86 53 00
Amity Milan, Italy 2-5097720 **Amersham Japan** Tokyo (03) 816 6161 **Amersham Nederland BV** 's-Hertogenbosch 073-41 82 25
Amersham — Norway branch Gjettum 02-54 63 18 **Amersham Sweden AB** Solna 08-734 08 00

. Reader Service No.5


nature
INTERNATIONAL WEEKLY JOURNAL OF SCIENCE
PREDICTING VOLCANIC ERUPTIONS
High-affinity nerve growth factor
The ring around supernova 1987A

nature
25 April 1991
Vol. 350 Issue no.6320

◄ Redoubt volcano (Alaska) in eruption on 21 April 1990. A mushroom-shaped tephra plume rises more than 8 km above sea level, from a pyroclastic flow on the north flank; a smaller, lighter-coloured plume rises from the summit crater. The view is across Cook Inlet. A combination of the 'materials science' method and seismic monitoring provides a promising way of predicting such explosive reactions. Page 695. Cover photo: Robert J. Clucas.

THIS WEEK . . . THIS WEEK . . . THIS WEEK . . .

NGF bound

The *trk* proto-oncogene has been found to be the complementary transmembrane molecule which, together with low-affinity nerve growth factor (NGF) receptor, forms the high-affinity receptor for NGF. Pages 678 and 660.

Ring found

A detailed study of the optical emission from the ring around supernova 1987A has made it possible to determine the velocity structure of the ring. The structure is revealed as a true physical ring — not a spheroid appearing as a ring because of an optical effect. Pages 683 and 654.

Food and community

The recent finding of widespread patterns in the shapes of food-webs, as well as advances in theoretical ecology, promise a much improved understanding of ecological complexity and community dynamics. Review Article, page 669.

Early symbionts

The discovery of a 50-million-year-old flower infested with a smut fungus is the earliest known record of an unusual symbiosis between flower and fungus. Page 698.

Seeing things

Psychophysical experiments with a new kind of stimulus allow insights into the process of visual 'filling-up', responsible for the way the natural visual blindspot is ignored. Pages 699 and 659.

Dangerous enhancer

The transcription factor NF-κB is known to be a potent activator of transcription from the human immunodeficiency virus (HIV) enhancer. On page 709, Bachelerie *et al.* show that chronic HIV infection of monocytes leads to induction of NF-κB and to increased HIV enhancer activity. News and Views, page 658.

Settling differences

For carbonaceous rocks and organic-rich sediments to be formed, organic matter must be preserved. The view that preservation is maximized in anoxic environments is now challenged. Radiocarbon dating of sediments from the Black Sea, indicates that the accumulation of organic sediments there is no faster than in the oxygenated open ocean. Page 692.

Behaving oddly

Neutron scattering provides evidence for anomalous behaviour above the transition temperature in the high-T_c superconductor $YBa_2Cu_3O_{7-\delta}$. The effects are larger than expected from elementary models. Page 690.

Short change

There has been much argument about the preference of major histocompatibility complex molecules for specific antigenic peptides. Schumacher *et al.* show (page 703) that MHC class molecules preferentially bind short peptides even if they are a minor component in a synthetic mixture, casting doubt on conclusions drawn from experiments using longer synthetic peptides.

Only adhere

The sialoglycoprotein CD43, which is defective in patients with immunodeficiency due to Wiskott–Aldrich syndrome, is reported to enhance the activation of transfected T cells, but only when its cytoplasmic domain is intact. Page 706.

The secret's out

The Commentary, 'Science and secrecy' by Walter Ormerod (page 551 of 18 April issue) did not appear on the contents page. This was not a further attempt to deny biologists access to centrifugation techniques developed by the military, as discussed in the article, but an error in the *Nature* office.

NATURE SAYS

Soviet progress depends on Gorbachev's survival ■ Cholera still with us ■ Software copyright unfair 639

NATURE REPORTS

European cooperative research ■ To Brussels ■ Nuffield on ethics ■ US research funding ■ Laboratory animals ■ Supernova neutrinos ■ Oceanography ■ Human Frontiers ■ Sandoz research deal ■ Drug patents ■ India's census ■ Great white sharks ■ Grants go electronic ■ German telecommunications ■ Gauss on money 641

CORRESPONDENCE

German unification ■ ■ Etcetera 648

COMMENTARY

Does climate still matter?
Jesse H Ausubel 649

NEWS AND VIEWS

Is Darwinism a thermodynamic necessity?
John Maddox 653
Supernovae: Running rings around SN1987A
Ph Podsiadlowski 654
Hotspots: Bermuda stretches a point
Randall M Richardson 655
Biopolymers: Light on molecular recognition
Robert Kaiser, Tim Hunkapiller & Leroy Hood 656
HIV: Tampering with transcription
Gary J. Nabel 658
Solid-state physics: Glasses come to order
Raymond Jeanloz & Quentin Williams 659
Neurobiology: *trking* neurotrophic receptors
Cliff Ragsdale & Jim Woodgett 660
Fusion energy: The moment of truth nears
Richard D. Petrasso 661
Daedalus: Dead heat 662

SCIENTIFIC CORRESPONDENCE

Underflying Brilliant Pebbles D C Wright & L Gronlund ■ **Is C_{60} stiffer than diamond?** R S Ruoff & A L Ruoff 663
Plastid genes and parasitic plants R M Wallsgrove ■ **Muscle damage in mdx mice** M J Jackson, A McArdle, R H T Edwards & D A Jones 664

BOOK REVIEWS

The Joy of Insight: Passions of a Physicist *by V Weisskopf* William H Press 665
Genes and Cancer *eds D Carney & K Sikora*; **Molecular Genetics and Cancer Diagnosis** *by J Crossman* Antony J Michalski ■ **Particle Physics and Inflationary Cosmology** *by A D Linde* Neil Turok 666
Nucleic Acids in Chemistry and Biology *eds G M Blackburn & M J Gait*
Richard I Gumport 667►

Nature® (ISSN 0028-0836) is published weekly on Thursday, except the last week in December, by Macmillan Magazines Ltd (4 Little Essex Street, London WC2R 3LF). Annual subscription for USAandCanada US$350 (institutional/corporate), US$135 (individual making personal payment). USA and Canadian orders to: *Nature*, Subscription Dept, PO Box 1733, Riverton, NJ 08077-7333, USA. Otherorders to *Nature*, Brunel Road, Basingstoke, Hants RG21 2XS, UK. Second class postage paid at New York, NY 10012 and additional mailing offices. Authorization to photocopy material for internalorpersonal use, or internal or personal use of specific clients, is granted by *Nature* to libraries and others registered with the Copyright Clearance Center (CCC) Transactional Reporting Service, provided thebasefee of $1.00 a copy plus $0.10 a page is paid direct to CCC, 27 Congress Street, Salem, MA 01970, USA. Identification code for *Nature*: 0028-0836/91 $1.00 + $0.10. US Postmaster send address changesto: *Nature*, 65 Bleecker Street, New York, NY 10012. Published in Japan by Nature Japan K.K., Shin-Mitsuke Bldg, 36 Ichigaya Tamachi, Shinjuku-ku, Tokyo 162, Japan. © 1991 Macmillan Magazines Ltd.

Working Smart in the 1990's

Introducing more than 500 products and services for your molecular biology needs

Gigapack® Packaging Extract – For the highest packaging efficiencies commercially available

Stratacooler®II and Stratacooler®Lites – Portable benchtop freezers designed to maintain subzero temperatures

Perfect Match™Polymerase Enhancer – Increases reaction specificity

StrataClean™Resin – Safe and convenient DNA purification

Big Blue™Mouse Mutagenesis Assay System – Transgenic technology for toxicity testing

Mammalian Transfection Kit – For optimum mammalian transfection efficiencies

FLASH™Prime-It™ – Highly sensitive chemoluminescent detection system

BaseAce™Vertical Sequencing Apparatus and Feathervolt™2000 Power Supply – For sequencing, footprinting, and other analyses

NucTrap™Push Columns – Fast probe purification system

Call for our Free Catalog.
1-800-424-5444

STRATAGENE®

Corporate Headquarters, USA – 1-800-424-5444
Telefax: 619-535-5430
Germany, Stratagene GmbH – (06221) 40 06 34
Telefax: (06221) 40 06 39
United Kingdom, Stratagene Ltd. – (0223) 42 09 55
Telefax: (0223) 42 02 34

Reader Service No.57

The Expendable Future: US Politics and the Protection of Biological Diversity
by R Tobin Stuart L Pimm 668

REVIEW ARTICLE

Food web patterns and their consequences
S L Pimm, J H Lawton & J E Cohen 669

ARTICLES

Medium-range order in the cation distribution of a calcium silicate glass
P H Gaskell, M C Eckersley, A C Barnes & P Chieux N&V 675

High-affinity NGF binding requires coexpression of the *trk* proto-oncogene and the low-affinity NGF receptor
B L Hempstead, D Martin-Zanca, D R Kaplan, L F Parada & M V Chao N&V 678

LETTERS TO NATURE

Velocity structure of the ring nebula around supernova 1987A
A P S Crotts & S R Heathcote N&V 683

The physical state of the intergalactic medium
X Barcons, A C Fabian & M J Rees 685

A nearby 37.9-ms radio pulsar in a relativistic binary system
A Wolszczan 688

Anomalous neutron scattering near the superconducting phase transition
N R Bernhoeft, P J Allen, D McK Paul, S M Hayden, P A Timmins & G G Lonzarich 690

Low organic carbon accumulation rates in Black Sea sediments
S E Calvert, R E Karlin, L J Toolin, D J Donahue, J R Southon & J S Vogel 692

Prospects for eruption prediction in near real-time
B Voight & R R Cornelius 695

A fossil smut fungus from the anthers of an Eocene angiosperm
R S Currah & R A Stockey 698

Perceptual filling in of artificially induced scotomas in human vision
V S Ramachandran & R L Gregory 699

Peptide selection by MHC class I molecules
T N M Schumacher, M L H De Bruijn, L N Vernie, W M Kast, C J M Melief, J J Neefjes & H L Ploegh 703

Enhancement of T-cell activation by the CD43 molecule whose expression is defective in Wiskott–Aldrich syndrome
J K Park, Y J Rosenstein, E Remold-O'Donnell, B E Bierer, F S Rosen & S J Burakoff 706

HIV enhancer activity perpetuated by NF-κB induction on infection of monocytes
F Bachelerie, J Alcami, F Arenzana-Seisdedos & J-L Virelizier N&V 709

Regulation of cellular responsiveness to inductive signals in the developing *C. elegans* nervous system
D A Waring & C Kenyon 712

Phosphorylation of two small GTP-binding proteins of the Rab family by p34^{cdc2}
E Bailly, M McCaffrey, N Touchot, A Zahraoui, B Goud & M Bornens 715

Inhibition of telomerase by G-quartet DNA structures
A M Zahler, J R Williamson, T R Cech & D M Prescott 718

N&V See News and Views

CLASSIFIED

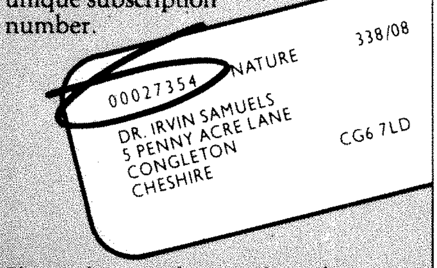
Professional appointments ■ Research posts ■ Studentships ■ Fellowships ■ Conferences ■ Courses ■ Seminars ■ Symposia Back pages

NEXT WEEK IN NATURE

Is there a natural limit to greenhouse effect? ■ Competition for mating partners ■ Mechanism of deep earthquakes ■ Science in Germany ■ Gene transfer in eukaryotic DNA viruses

SUBSCRIPTION ENQUIRIES

Your mailing label contains your unique subscription number.



Please make a note of your number and quote it whenever you contact us about your subscription.

Annual Subscription Prices (including Annual Index)

UK		£136
Rest of Europe		£145
USA & Canada		US$350
Australia & NZ	Airspeed	£165
	Airmail	£215
India	Airspeed	£132
	Airmail	£215
Japan		¥95000
Rest of World	Surface	£149
	Airmail	£215

Orders (with payment) & subscription enquiries to:

USA & Canada
Nature
Subscription Dept
PO Box 1733
Riverton
NJ 08077-7333 USA

UK & Rest of World
Nature
Subscription Dept
Brunel Road, Basingstoke
Hants RG21 2XS, UK
Tel: 0256 29242

(The addresses of Nature's editorial offices are shown facing the first editorial page)

Japanese subscription enquiries to: Japan Publications Trading Company Ltd, 2-1 Sarugaku-cho 1-chome, Chiyoda-ku, Tokyo, Japan. Tel: (03) 3292 3755

Personal subscription rates: These are available in some countries to subscribers paying by personal cheque or credit card. Details from:

USA & Canada
Nature
65 Bleecker Street
New York
NY 10012, USA
Tel: (212) 477-9600

UK & Europe
Marketing Department
Nature
4 Little Essex Street
London WC2R 3LF, UK
Tel: 071-872 0104

Back issues: UK, £3.00; USA & Canada, US$15.00; Rest of World, £4.00 (surface), £5.00 (air)

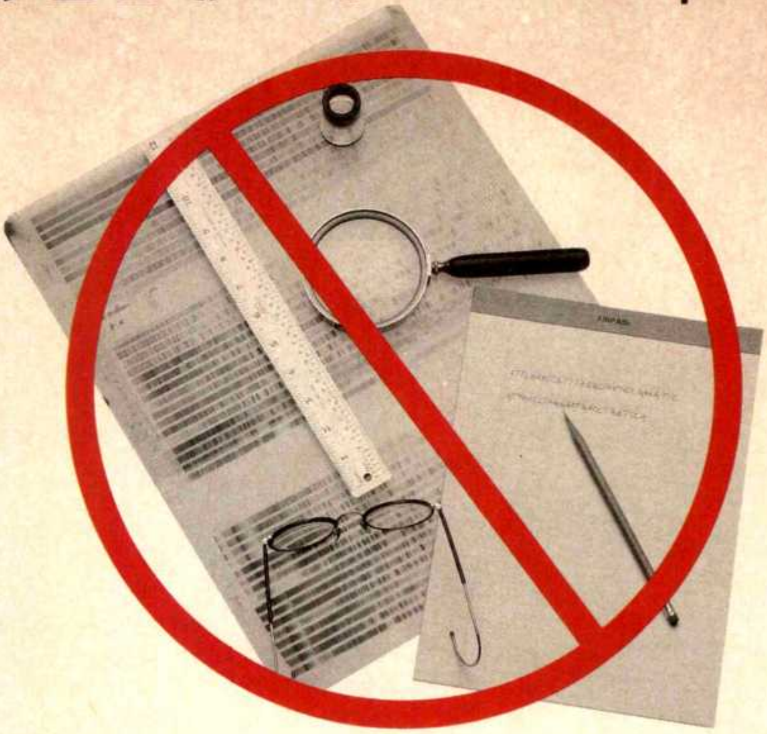
Binders
Single binders: UK, £5.25; USA $10.95; Canada $12.45; Rest of World, £7.75. Set of four: UK, £19.50; USA $39.95; Canada $45.95; Rest of World, £28.00

Annual indexes (1971-1989)
UK, £5.50 each; Rest of World £7.00; USA & Canada US$25.00

Nature First Issue Facsimile
UK £3.50; USA/Canada US$25.00; Rest of World £6.00

Nature in microform
Write to University Microfilms International, 300 North Zeeb Road, Ann Arbor, MI 48106, USA

CAUTION: This May Change The Way You Read DNA Sequencing Films



The Discovery Series™ Model DNA 35 Automatically Reads DNA Sequencing Films

Hardware, Software and how it will change the way you do DNA Sequencing Reading.

To compete in today's world, you can use the old tools...or...choose today's technology. The **Discovery Series™ model DNA 35.**

- It gives you the power to scan, read, edit and compare 3,000 bases in 30 minutes.
- It fits on a desktop or lab bench.
- The new DeskTop™ scanner provides the image to work with.
- **DNA Code**® **v2.0.** A new software release with more sequence reading performance to turn *your* image into data.
- **New 16 MIPS UNIX**® minicomputers work as fast as you want.
- It also comes with **Quantity One**® – 1-D gel and dot/slot blot software.

Scan your film in seconds at 63.5 micron resolution and watch the digitized image flow onto your computer screen in real-time. The combination of our new DNA sequence reading software, a new high-resolution scanner, and UNIX® based computers gives you real-life, magnified graphics to work with. Automatic base-calling and interactive editing tools finish the task.

1-D gels, dot & slot blots? Yes, the model DNA 35 can quantitate all types of 1-D gels and films.

You're doing more than DNA Sequencing. That's why the DNA 35 also comes with **Quantity One**,® the software package for the quantification of all types of 1-D applications and dot/slot blots.

UNIX® is a trademark of AT&T Laboratories. Quantity One® and DNA Code® are trademarks of PDI, Inc.

Application	Software Package
DNA sequence reading	DNA Code®
1-D gel quantification	Quantity One®
Dot and slot blot analysis	Quantity One®

Call PDI toll-free today for more information.

Call **1-800-777-6834** (USA only) to set up a demonstration of The Discovery Series™ model DNA 35 sequencing DNA in *your* lab. We look forward to working with you to turn *your* data into results.

pdi

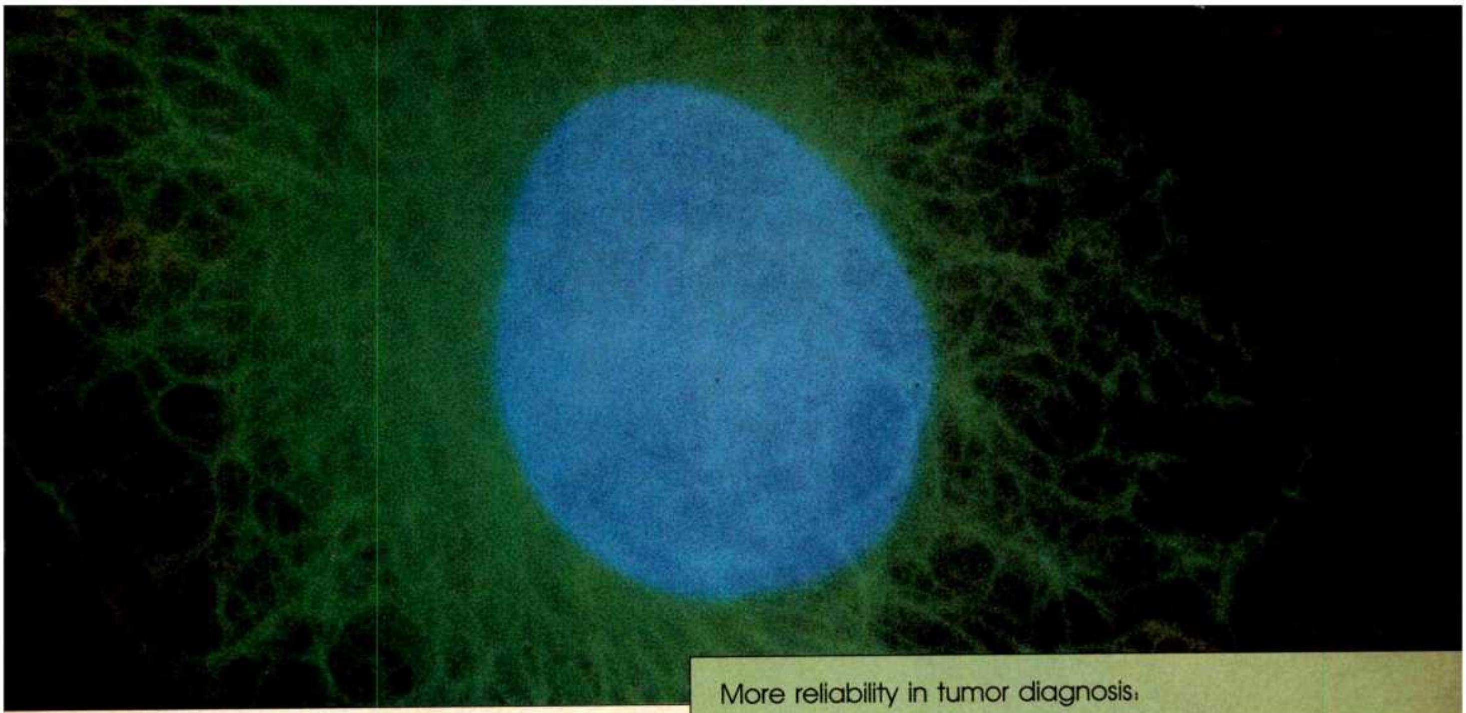
formerly protein databases inc

protein + dna imageWare systems ℠

405 Oakwood Road, Huntington Station, New York 11746
Phone: 516-673-3939, 1-800-777-6834, Fax: 516-673-4502

Reader Service No.594

In the Benelux countries: DALTON, bv., PO Box 154, NL 5140 AD Waalwijk, The Netherlands. Tel: (0)4160-31225, Fax: (0)4160-41265
In Japan: Toyobo Co. Ltd., Osaka, Japan, Tel: 06-348-3786, Fax: 06-348-3322
In Germany, Austria, Switzerland and Eastern Europe: Sud-Laborbedarf, GMBH, D 8035, Gauting, FRG, Tel: 089 850 6527, Fax: 089 850 7646



More reliability in tumor diagnosis:

Differentiate between cytoskeletal proteins

PROGEN cytoskeletal antibodies:
Developed and manufactured inhouse

Quality and service are assured by:

- our worldwide cooperation with leading research institutes
- the unusually wide range of products available
- the consistent high quality standard of all PROGEN products
- the extra reliability we add to your work

Our Research Products catalog shows you all the unique products which are only available from PROGEN. Send us the coupon – to make sure of your copy

PROGEN Biotechnik GmbH, Im Neuenheimer Feld 519,
D-6900 Heidelberg, Tel.: 0 62 21 / 40 35 - 0, Tx.: 4 61 124,
Fax: 0 62 21 / 40 35 35

Research for mankind – PROGEN

Reader Service No. 308

Yes, please send me your catalog.

Name ____________________

Address ____________________

____

PROGEN

The world's most intensively cited
leukemia journal, now published by Macmillan

LEUKEMIA

EDITORS

C. Nicole Muller-Bérat, Denmark
William M. Crist, USA   Edward S. Henderson, USA
Mel F. Greaves, UK

CLINICAL STUDIES
■
CELL BIOLOGY
■
MOLECULAR HEMATOLOGY

- Differentiation
- *In Vitro* Models
- Molecular Pharmacology
- Membrane Markers
- Oncogenes
- Gene Therapy
- Enzymatic Markers
- Stem Cells
- Virology
- Biological Modifiers
- Bone Marrow Transplantation
- Therapeutic Trials
- Cell Cycle
- Growth Factors
- Chromosomes

FROM RECENT ISSUES OF LEUKEMIA

OPEN FORUM:

Remarkably Long Survival of a Patient with Ph^1-Positive Chronic Myeloid Leukemia and 5′ bcr Rearrangement *B. Johansson, F. Mertens, T. Fioretos, S. Heim, U. Kristoffersson, N. Mandahl, C.R. Bartram & F. Mitelman*

Cure of Acute Myelocytic Leukemia in Adults: A Reality *R.B. Geller, R. Saral, J.E. Karp, G.W. Santos & P.J. Burke*

CONGRESS LETTER:

Chronic Myelogenous Leukemia — The Unfolding Saga *J.M. Goldman, G. Grosveld, D. Baltimore & R.P. Gale*

COMMENTARY:

The Sellafield Childhood Leukemia Cluster: Are Germline Mutations Responsible? *M.F. Greaves*

ORIGINAL ARTICLES:

Induction of Genes for Transcription Factors by normal Hematopoietic Regulatory Proteins in the differentiation of Myeloid Leukemic Cells *Y. Shabo, J. Lotem & L. Sachs*

Granulocyte Colony-Stimulating Factor, not Granulocyte-Macrophage Colony-Stimulating Factor, cooperated with Retinoic Acid on the Induction of Functional N-Formylmethionyl-Phenylalanine Receptors on HL-60 Cells *A. Sakashita, T. Nakamaki, N. Tsuruoka, Y. Honma & M. Hozumi*

Serum Interleukin-2, Soluble IL-2 Receptors and Tumor Necrosis Factor-Alfa levels are significantly increased in Acute Myeloid Leukemia Patients *G. Cimino, S. Amadori, M.C. Cava, V. De Sanctis, M.C. Petti, O. Di Gregorio, C. Sgadari, L. Vegna, G. Cimino & F. Mandelli*

Effects of Recombinant Human Granulocyte Colony Stimulating Factor and Granulocyte-Monocyte Colony Stimulating Factor on in Vitro Hemopoiesis in the Myelodysplastic Syndromes *A. Nagler, N. Ginzton, R. Negrin, D. Bang, T. Donlon & P. Greenberg*

Is the mdr 1 Gene Relevant in Chronic Lymphocytic Leukemia? *J.A. Holmes, A. Jacobs, G. Carter, J.A. Whittaker, D.P. Bentley & R.A. Padua*

Published on behalf of the Sven-Aage Killmann Foundation

Reader Service No.16

Subscription Rates

Monthly
ISSN 0887 6924

ONE YEAR	
Institutional	£202
Individual	£101
TWO YEARS	
Institutional	£404
Individual	£202

The individual rate is only available if you are paying with your **personal** cheque or credit card.

SEND FOR YOUR FREE SAMPLE COPY

To Dionne Stocking, Macmillan Press Ltd, Houndmills, Basingstoke
RG21 2XS, UK.

☐ Please send me a **FREE** sample copy of Leukemia

Name ____________________

Position ____________________

Address ____________________

____

____

Postal/Zip Code ____________ Country ____________

NAT

M

CLIP THIS COUPON

BIOTECH USA®
PHARMBIOTECH
AGBIOTECH
BIOLAB
BIOBUSINESS
SEPTEMBER 25-27, 1991
PHILADELPHIA CIVIC CENTER
PHILADELPHIA, PA

For more information on BIOTECH USA '91 -
the innovative conference & exhibition
for the fields of pharmaceutical and agricultural biotechnology -
mail this coupon to: BIOTECH USA, c/o CMC, 200 Connecticut Avenue,
Norwalk, CT 06856-4990 or FAX to 203-838-3710

I am interested in: ☐ Attending ☐ Exhibiting

Name ______________________________

Title ______________________________

Company ______________________________

Address ______________________________

City __________________ State ______ Zip __________

Phone (____) __________________ Country __________________

Fax ______________________________

B4

AND SEND IT TODA

NEW

Nature offers affordable reprints of the articles you need

Now both academics and companies can benefit from Nature's quality reprint service

Academics

- Distributing reprints at your lectures and seminars is the best way to involve your audience.

Companies

- Keep key individuals up-to-date with essential scientific developments by ordering reprints for important meetings.
- Prove your organisation is a world leader by sending important clients reprints of articles by company scientists.

nature

From 1 page in black and white to 8 pages in full colour, Nature has reprint options to suit your needs and your budget. For example 100 copies of a 4 page black and white reprint costs only £150.

To receive further information simply complete the form below and return it to the address indicated.

Information Request form

☐ Yes please send me price details for Nature's new reprint service.

Name ______

Address ______

______

______

Postcode/Country ______

Please return this form to Elisabeth Shaw, Nature Marketing, 4 Little Essex Street, London WC2R 3LF, UK

NATURE EDITORIAL OFFICES

LONDON: 4 Little Essex Street, WC2R 3LF
Telephone: (071) 836 6633 Fax: (071) 836 9934 & 240 6930

Editor: John Maddox

Managing Editor: Nuala Moran

Editorial Staff: Peter Aldhous, Philip Ball, Maxine Clarke, Christopher Coath, Rosalind Cotter, Alexander Gann, Laura Garwin, Henry Gee, Rory Howlett, Tim Lincoln, Philippa Lloyd, Anne Martin, Lindsay Matthews, Geoffrey North, Roland Pease, Miranda Robertson, Teresa Scranney, Peter Tallack, Richard Vile and Charles Wenz.

Art & Design: Nicola Barker, Jacqueline Dowdall and Stephen Sullivan

Administration: Mary Sheehan, Lucy Bending, Tamara Boake, Joanna Casale, Tania Coad, Jacqueline Hood, Barbara Izdebska, Ruth Lefèvre, Johanne Peters, Helen Slack, Anne Vericel and Rosalind White

WASHINGTON: 1137 National Press Building, DC 20045
Telephone: (202) 737 2355 Fax: (202) 628 1609

Deputy Editor: Barbara J Culliton

Editorial: Christopher Anderson (News), Kevin Davies (Assistant Editor, Biology), Diane Gershon (Product Review and News), David Lindley (Associate Editor, Physical Sciences) and Robert Pool (News Editor)

Administration: Penelope Walker, Kathryn Bielenberg and Laura Bellows-Ancasi

MUNICH: Sandstrasse 41, 8000 München 2
Telephone: (089) 527036 Fax: (089) 523 2222

Munich Correspondent: Steven Dickman

TOKYO: Shin-Mitsuke Building (4F), 3–6 Ichigaya Tamachi, Shinjuku-ku, Tokyo 162
Telephone: 03 3267 8751 Fax: 03 3267 8746
Publisher & Tokyo Correspondent: David Swinbanks
Promotion Manager: Koichi Nakamura
Japan Advertisement Manager: Phillip Hamill
Administration: Rinoko Asami

Managing Director: Ray Barker
Editorial Director: Mary Waltham
Marketing Manager: Ross Sturley
International Advertisement Manager: Fiona Anderson
European Sales Manager: Daryl Rayner
Sales Executives: Angela Burry and Emma Hipwell
Classified Advertisement Manager: Julie Skeet

NEW YORK OFFICE: 65 Bleecker Street, New York, NY 10012
Telephone: (212) 477 9600 Fax: (212) 505 1364
President: Andy Sutherland
Vice Presidents: Marion Delaney and James A. Skowrenski
American Advertising Manager: Ralph Frakes
Classified Advertising Manager: Marianne Ettisch
Advertising Consultant: Henry Dale

CANADA: Display & Classified: Peter Drake, 17 Pine Crescent, Toronto, Ontario M4E 1L1. Telephone: (416) 690 2423

DISPLAY ADVERTISING REPRESENTATIVES

New York: Nature Office
Chicago: Didier & Broderick, Inc. (708) 498 4520
West Coast and Southwest: Lynne Stickrod (415) 781-3801
East Coast: Glenn Melvin (212) 477 9608
France: Clare Newell Tel. (1) 48 87 24 43/Fax (1) 48 87 46 71
Switzerland: Verena Loewenthal, Interpress, CH-8103 Unterengstringen, Zurich (01) 750 5511/Fax (01) 750 2210
Israel: Daniel Zimet, Zimet Advertising & Publishing (03) 261653/Fax (03) 253391
Netherlands and Belgium: Adele Struyck, Astra, Netherlands (03431) 1702
Scandinavia: Andrew Karnig, Andrew Karnig & Associates, Sweden (08) 4400 05
Germany and Austria: Sabine Fürst Tel. (089) 524081/Fax (089) 523222

Nature® ISSN 0028-0836
Registered as a newspaper at the British Post Office
© 1991 Macmillan Magazines Ltd
Vol. 350 No. 6320 25 April 1991

NATURE VOL 350 nature 25 APRIL 1991

Mikhail Gorbachev's tightest corner

This week and those immediately ahead should see at least some decisions made, or alternatively taken by default, about the future of the Soviet Union and its leader, Mr Mikhail Gorbachev.

Last week's first visit to Japan by a Soviet head of state was predictably a disappointment, given that the Soviet Union had said in advance that it would not concede Japan's case for the restoration of the disputed Kurile islands, annexed in 1945, but Mr Mikhail Gorbachev can be pleased about at least one feature of the visit: his enemies at home did not seize on his absence as an opportunity to depose him from one or other of his powerful positions as president of the Congress of Deputies and secretary general of the Soviet Communist Party. All too often, people in his position have found themselves deposed while glad-handing in foreign capitals. But Gorbachev cannot carry on much longer unless he manages to break out of the pattern of inanition into which he has slumped in the past few months.

The most tangible symptoms of his difficulty are economic. Last week, the government allowed that industrial production in the Soviet Union has fallen in a year by 8 per cent, comparable with the extent to which Western economies were depressed during the 1930s. At the same time, exports have fallen by two-thirds and the government is quickly running through the stock of freshly printed roubles it had hoped would last for the whole year. The prospect of an economic catastrophe on a scale even greater than the appalling situation of the past year stares the Soviet Union in the face.

Opportunity

What can be done? On this narrow front, Gorbachev's weakness is that he has let slip past opportunities to put the Soviet economy to rights. The 'price reforms' introduced last month, which are not really reforms but merely new and higher prices centrally decreed, were first planned in 1987. Then, they might have worked. Now, after four years of needless delay, they seem merely to have precipitated a series of strikes that will further disrupt industrial production.

More recently, a year ago, Gorbachev blew hot and cold (in that order) about the plans for the more radical reform of the economic structure being canvassed by people such as Academicians S. S. Shatalin and A. G. Aganbegyan. Having encouraged the idea, fostered by Mr Boris Yeltsin, that the rudiments of a market economy should be introduced as the first step in a whirlwind upheaval lasting just 500 days, Gorbachev first hijacked the idea and then abandoned it. Not surprisingly, that proved a recipe for losing friends and helpers. Now, towards the end of a miserable winter, even the struggling free-market cooperatives have been further constrained by decree from above. Even if Gorbachev now wished to resuscitate those lost opportunities, it is doubtful whether the Communist Party and the *soyuz* faction in the Congress would let him.

Separation

At this late stage, in any case, an exclusively economic reform, however radical, will not put the Soviet Union and its several increasingly independent parts to rights. Assertions of separation by the three Baltic states and Georgia remain in a kind of limbo, while the relationship between the old command centre — the Central Committee and the Soviet government — with the republics and especially Yeltsin's Russian Federation, are similarly unresolved. Meanwhile, the fabric of an ingenious society hardy enough to have survived 70 dreadful years is being quickly eroded by people's growing perception that they must fend for themselves. In all the circumstances, the surprise is not that Soviet science seems to have benefited little from *glasnost* (which persists), but that some heroic people are still to be found working at the bench. For how much longer?

The coming weeks should settle some of these undecided issues. For Gorbachev, and for the Soviet Union as a whole, this Wednesday's plenum of the Communist Party will be crucial. From the outset, Gorbachev's ambivalence about the party's role in the Soviet scheme of things has been a source of confusion. His chief constitutional innovation has been to create the Congress of Peoples' Deputies, in principle at least an elected parliament, but the party retains the right to nominate government ministers (who are then approved, or otherwise, by the Congress).

Four years ago, Gorbachev might have risked resigning his post as general secretary, relying on the compliance of a discredited organization to yield governments that would have been acceptable or, even better, transferring that crucial function from the party to the Congress. His failure to grasp that nettle probably explains why he has become the prisoner of party opinion on centralism, the hankering after the concept of a planned imperial state to which two tiny islands off the coast of Japan are indispensable. Now, resignation would risk confrontation between the party and centrifugal nationalism on the one hand and democratic liberalism on the other. That is one of several ways in which civil war might start. The best hope is that Gorbachev stays on, and is allowed to do so, at least for the time being.

But that can be only half the battle. There also needs to be a plan to make the Soviet Union function. Those who at the outset welcomed Gorbachev's *glasnost* and his plans for *perestroika*, as if dismayed at the degree to which he has been weakened by the ferocity of their protests at what seemed to be backsliding, are now offering the olive branch of some kind of coalition government. If it could be realized, it might just work. But the chances are only small, while those of breathing new life into one of the discarded economic plans have been much reduced by the widespread sense of having been monkeyed with that Soviet people now widely share. □

Soft on software

Europe, which plans to protect software against piracy, should now give some thought to those who buy it.

EARLIER this month, the European Commission did the decent thing by the international software industry by requiring member states to adopt uniform copyright laws on commercial software. Both equity and commercial prudence require nothing less. To copy a piece of commercial software illicitly is theft of a literal kind, where the victims are those who have invested skill, time and money in the development of a useful set of computer instructions. The uniform extension of copyright legislation to software is also commercially prudent; otherwise, the supply might dry up. Software manufacturers say they are pleased with the development, but also (rightly) that enforcement is not child's play.

So far, so good. But before tears are shed for software manufacturers, some thought should be given to their licit customers, and to the terms on which software packages are sold. The use of the term copyright suggests an analogy with books, but the analogy is inexact in at least two ways. First, a book (at least from a reputable publisher) is a finished product that is unlikely to be substantially revised for at least some time; software packages, on the other hand, are published almost breathlessly, with 'version numbers' growing steadily in each of their two significant digits. Second, books enjoy well-understood economies of scale, with the result that publishers price their products so as to make money if their expectations are requited, but otherwise lose. By contrast, the intrinsic value of a software package is negligible compared with the selling price.

These circumstances put software purchasers at a disadvantage compared with mere book-buyers. For one thing, having bought a software package advertised as serving some stated purpose, they must then pay extra to upgrade to a new version that will actually do the job. Second, they derive no benefit from economies of scale. Whereas a successful book will probably appear eventually more cheaply as a paperback, a successful and widely used software package will keep its original high price until some other manufacturer is able to achieve the same results with a separately written set of codes. And as things are, it is not possible for people to buy only part of a software package, perhaps to carry out only one of many functions. The European Commission, which is often fierce in its execution of anticompetition policy, should now look at the other side of the software coin. □

Cholera marches on

The outbreak of cholera in northwestern South America could cast a long shadow on the world.

CHOLERA is always with us. That is literally the case. The causative organism, *Vibrio cholera*, is widely spread among the human population, but the enterotoxin that the organism secretes is likely to cause intestinal disease only when there are as many as 100 million organisms in the gut. The consequence is that cholera organisms can be widely spread yet cryptic in a population until some external agency, usually a deterioration of hygiene, causes them to multiply. In the early decades of the nineteenth century, the disease was commonplace in major European cities. Even now, it is endemic in cities such as Calcutta, where sporadic cases repeatedly crop up. But what is to be made of the outbreak of cholera since January in the South American states of Chile, Peru, Ecuador, Colombia and, now, some parts of Brazil?

More than 150,000 people have been affected in Peru alone, of whom more than 1,000 have died. It seems to have been recognized by the governments of the region, which met last week in Bolivia, that the necessary remedies are those well understood for more than a century — supplies of clean water, foodstuffs similarly free from contamination and better sanitation. But this simple recipe is more easily stated than made reality. Otherwise, the present outbreak, which is but the latest manifestation of a pandemic that began in Indonesia in 1961 and which caused 150,000 cases in Africa a decade later, might have been avoided.

Dr John Wood, the London physician, made himself a legend when, in 1854, he removed the pump-handle from a polluted communal water supply in the City of London's Broad Street, bringing a cholera outbreak quickly to an end, but plainly there can be no such simple solution in the vast region already affected in South America. Public education in the principles of personal hygiene is the only ready expedient. The nightmare — made explicit at the weekend by an official of the Panamanian Health Organization — is that the outbreak might spread to the great cities of the Amazon basin and, from there, to Brazil's shameful shanty towns around Saõ Paolo and Rio de Janeiro.

There is an even more daunting prospect. Iranian health officials are already alarmed by cases of cholera in southern Iraq, among the people recently afflicted by the Gulf War and its aftermath. (Whether cases are officially reported depends on the willingness of governments to take such invidious action.)

Already fragile public health arrangements have deteriorated and, worse, the tragic displacement of people from their homes has made them dependent on makeshift and insecure sanitary systems. In general, cholera is an attribute of poverty, and of the lack of public investment in water supplies and sanitation, but social upheavals like those to be expected in the Middle East in the next several years are also precipitating causes. We shall hear a lot more of cholera before much more time passes. □

Compromise averts Framework fiasco

Five research programmes back on track
A continuing power struggle over policy

London

THE warring institutions of the European Communities (EC) have managed to patch together a compromise that should prevent the withdrawal, announced by the European Commission last month, of five planned research programmes (see *Nature* **350**, 177; 21 March 1991).

The withdrawal of the research programmes, the only five among the 15 of the EC's third five-year Framework research budget that are nearing final approval, would have delayed any spending of the budget of about £4,000 million (5,700 million European Currency Units) until at least late 1992. The third Framework was intended originally to run from 1990 to 1994.

Although the immediate crisis has been resolved, the third Framework remains badly behind schedule, and further political upheaval is possible. Most of the arguments over EC research stem from the perennial struggle for power between the ministers from EC member states, the European Parliament and the Commission, which is the EC's civil service. With the EC's constitution due for redrafting, these battles are likely to intensify, as the member states debate plans for greater political unity.

The recent dispute over the research budget began when the Commission, backed by the European Parliament, said it was withdrawing the five programmes because the research ministers from the EC member states had altered the programmes from the Commission's original proposals, and had ignored amendments suggested by the Parliament. But the move was seen by many as a calculated act of defiance by the Commission and the Parliament, using the EC's research programme as a political battleground to press their case for increased power over EC policy — which is now dominated by the decisions taken at the occasional meetings of ministers from the EC states.

Jacques Delors and Enrique Baron Crespo, presidents of the Commission and the Parliament respectively, and Luxembourg's research minister Rene Steichen last week signed an agreement resolving the differences over the five research programme proposals, which is expected to be endorsed this week at meeting of EC research ministers.

Both sides claim to have given away little in the compromise. But some observers in Brussels believe that Delors, having made a political point, has now decided to leave the EC's research programmes alone.

The Commission and Parliament had disagreed with the ministers over a number of points concerning the management of the five research programmes. The main concession made by the ministers is to weaken some of the management committees that will oversee the programmes in the third Framework (not just the five disputed programmes) — so allowing the Commission more freedom to control research programmes.

The five threatened programmes, in environmental research, marine science and technology, life sciences, technologies for developing countries, and two programmes in telecommunications, should now be finally adopted in June, and the first grants may be awarded early next year. But the delays introduced by a series of skirmishes between the member states' ministers and the Commission mean that the grant spending under the rest of the programmes within the third Framework is unlikely to begin before mid-1992. This makes a mockery of the original plan to review the progress of each of the research programmes during 1992, halfway through the third Framework.

Nevertheless, Gordon Adam, vice chairman of the Parliament's Energy, Research and Technology Committee, believes the crisis of the past month may have had one positive effect. To forge the compromise, research ministers and members of the European Parliament have been forced to communicate with one another. Adam hopes the precedent will lead to a more open dialogue between the two groups.

But a further row over EC research is likely. A Commission proposal to have future Framework budgets and the details of their component programmes approved in a single step (see *Nature* **350**, 101; 14 March 1991) has been included in a draft revision of the EC's constitution, now being circulated around the EC. The draft has been produced by the intergovernmental conference that is considering plans for greater EC political unity. At present, the proposed outlines of individual research programmes are subject to scrutiny by research ministers and the Parliament after the overall Framework budget is agreed. If each Framework was approved in a single step, the Commission would have far more influence over EC research than is now the case.

Given the delays that have plagued the approval of the programmes that make up the third Framework, some EC states are expected to support the move to streamline the process. But British officials, fearing any extension of the Commission's power, will oppose the plan. **Peter Aldhous**

EUROPEAN RESEARCH

Setting up shop in Brussels

London

RESEARCH funding agencies from the European Communities (EC) member states are stepping up their presence in Brussels, anticipating an increase in the proportion of research spending by European governments coming through the EC.

The five British research councils last week merged their Brussels office with that of the British Council, which has been speaking with the EC on behalf of a number of British universities. The new UK Research and Higher Education European Office aims to ensure that UK scientists are able to compete effectively for EC research grants.

British scientists — aided by the research councils' presence in Brussels — currently earn more in EC research grants than the UK government pays into the various EC research programmes. The French and Germans have so far been less sucessful in winning EC funding, but now seem to be following the British example. French research funding agencies opened a Brussels office last month, and the Germans are soon to follow suit. P.A.

RESEARCH ETHICS

Nuffield takes the lead on bioethics

London

THE Nuffield Foundation, a large British research charity, will set up the first UK committee to consider the broad sweep of ethical issues thrown up by advances in biological research. The Nuffield Council on Bioethics will be chaired by Sir Patrick Nairne, formerly a senior civil servant at the UK Department of Health and Social Security, and will have a budget of some £150,000 a year.

David Shapiro, from the Nuffield Foundation's bioethics initiative, expects that the Nuffield Council will concentrate initially on the ethical questions surrounding new techniques in molecular biology, including genetic screening, and on publishing reports of its work. A majority of the council's members will be neither professional scientists nor medical doctors, Shapiro says.

The Nuffield Council breaks with the British custom of setting up separate *ad hoc* committees to tackle different ethical issues — for example, the Interim Licensing Authority for human embryo research, set up by the Medical Research Council and the Royal College of Obstetricians and Gynaecologists.

Perhaps the closest parallel for the new body is the French National Consultative Ethics Committee for the Life Sciences and Health, set up in 1983 — except that the French body is supported by public funds, rather than a charitable foundation. P.A.

RESEARCH FUNDING

Cuts coming on overheads

Washington

A CAP on the indirect costs of research, only a rumour two weeks ago, now seems nearly certain as US government officials and congressional leaders rush to respond to the overcharging scandal that is striking a growing number of US universities.

At a series of congressional hearings last week, Bush Administration officials launched a preemptive strike against feared congressional initiatives by proposing their own across-the-board caps on indirect costs. A task force on the subject chaired by Bernardine Healy, the new director of the National Institutes of Health (NIH), is seriously considering limits on total costs as well as restrictions on the portion of those costs that cover administrative expenses.

Richard Kusserow, inspector general of the Department of Health and Human Service (HHS) and a member of Healy's task force, testified that capping indirect costs at 50 per cent of direct research costs would save some $125 million each year, which could fund about 1,000 new research grants at NIH. Although 50 per cent is about the current average for indirect costs, declaring it an absolute ceiling would force universities above the average to lower their rates, resulting in an overall reduction in cost to the federal government — a reduction that universities will have to make up from their own resources.

Other proposals also remain under active consideration by the Healy panel and the White House Office of Management and Budget, although they are seen as less likely than the option of capping indirect costs. These alternatives include 'block grants' to universities, in which researchers and administrators would decide between themselves how much should go to direct and indirect costs, as well as grants awarded directly to researchers, who would then negotiate with their administrators what proportion would go to indirect costs.

Five years ago, the federal government proposed and nearly succeeded in winning a cap on the administrative portion of indirect costs, following the recommendations of a report by D. Allan Bromley (now director of the White House Office of Science and Technology Policy) and industrialist David Packard. At the time, such a cap would have saved $85 million per year — perhaps half of what a similar measure might save today in the larger NIH budget.

Last week, that proposal was resurrected when Kevin Moley of HHS suggested at least a 26 per cent cap on administrative indirect costs, which currently make up about half of total indirect costs. Given the accounting reforms since 1986, a ceiling on administrative costs might even be profitably lowered further, Moley told a congressional committee. HHS is also said to be considering a 20 per cent level.

Although that sort of proposal had full federal support in 1986, Congress, under university pressure, put language in its appropriations bill to prohibit it. This time, however, in the midst of an indirect-cost scandal, Congress seems unlikely to offer much opposition. Indeed, there is an unmistakable groundswell of determined — if somewhat undirected — congressional enthusiasm for cost cuts.

Science supporters fear that congressional appropriations committees, faced with deficit troubles and a host of competing financial demands, may simply reduce the NIH budget in anticipation of the savings from whatever cost-control plan is finally adopted. That money would presumably go to fill needs elsewhere in the US budget.

Science supporters in Congress are anxious to convince colleagues of the da gers of hasty cuts. “Most members of Co gress”, says George Brown (Democrat, Ca fornia), chairman of the House Scien Committee and strong research supporte “don't understand the issue and don't unde stand why the money [saved by capping inc rect costs] should go back into science. the possibility of a cap dawns, some of us w be trying to educate them.”

Meanwhile, university administrato fearing that the issue will soon be out of th hands, are trying to be seen as responsibl The Association of American Universiti last week told congressional leaders that has asked a panel of university presidents visit Washington and meet federal offici “to correct errors which call into questi our commitment to contain and monit prudently and responsibly the costs of co ducting sponsored research.”

Christopher Anders

ANIMAL RESEARCH

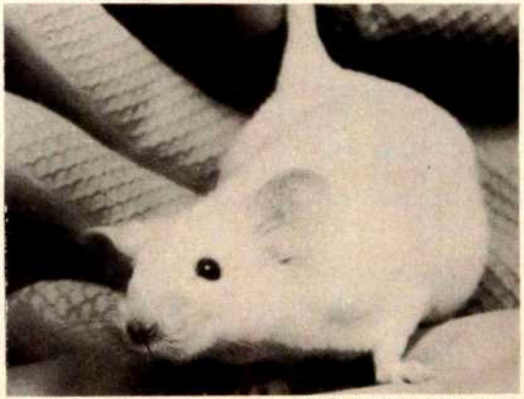
Court puts rules in question

Washington

A US COURT victory earlier this month by an animal-welfare organization has opened the door to a legal battle over federal restrictions on the care of rats, mice and birds in research, as well as a possible attack on the rules covering primates, dogs and cats.

The suit, filed in June last year by the Animal Legal Defense Fund, sought to force the US Department of Agriculture (USDA) to include rats, mice and birds in its animal care and housing regulations, which govern all US laboratory animals.

A clause in the 1971 congressional act that compels USDA to protect the welfare of laboratory animals gives the administrator of the agency the authority to determine what is and is not to be defined as a research animal. To simplify its enforcement, USDA has chosen not to define rats, mice and birds as 'animals' for regulatory purposes. The agency argues that including them would greatly increase its administrative workload without greatly improving their welfare, as most laboratory rat and mouse enclosures are already constructed in compliance with the accepted guidelines of the National Institutes of Health.



Is this laboratory mouse an animal? USDA says no.

In its decision earlier this month, the District Court for the District of Colum ruled that the animal defence group has t right to sue because it had been prevent from distributing information on laborat rats, mice and birds by the USDA decisi Because USDA does not collect informati on the animals and report it to Congress, t group is unable to pass the information on its members, and is prevented from carryi out one of its primary functions, the court cided.

Known as 'informational standing', t ruling appears to open the door to other la suits by animal-welfare organizations. Ma previous legal challenges by activists fail when the organizations were unable to pro to the court's satisfaction that they had be materially damaged by government actic an essential requirement for legal standin

Other animal organizations, including t Animal Welfare Institute, are closely watc ing the suit, with an eye on the newly releas USDA regulations on primates, dogs a cats (see *Nature* **349**, 641; 21 Februa 1991). Activists believe that the new rul which give laboratories great fle bility in determining the details of e closures and exercise regimes, are violation of congressional inte Now, they are considering suing.

The district court will turn its atte tion next to the merits of the anim defence fund's case. If it wins outrig USDA could be forced to include ra mice and birds in its regulations. T would more than double the num of facilities the agency must inspe and could cost several million doll each year in additional regulatory e penses, according to USDA calcu tions.

Christopher Anders

NEUTRINO ASTRONOMY

Italy looks to next supernova

Mont Blanc

For Oscar Saavedra and his colleagues at he Italian national research council (CNR) Istituto di Cosmogeofisica in Turin and the Soviet Academy of Sciences, the 1987 supernova in the Large Magellanic Cloud came eight years too early.

Four years ago, the Italian/Soviet collaboration was the first to report a burst of neutrinos from SN1987A, which they had detected at their underground observatory at Mont Blanc, 2,000 metres below the Italian/French border in an emergency bay off the Mont Blanc road tunnel. That neutrino sighting, however, is now thought by most physicists to have probably been due to background noise and the detectors apparently missed a real neutrino burst that occurred several hours later.

So now INFN, the Italian national institute for nuclear physics, is building a larger neutrino detector at the Gran Sasso underground physics complex about 650 km southeast of Mont Blanc. This Large Volume Detector (LVD), which is expected to be completed in three to four years, might well have helped to establish whether the Mont Blanc detection was a real neutrino event linked to SN1987A if the LVD had existed at the time, Saavedra says.

Standard astrophysical theory holds that a collapsing star, before it explodes as a supernova, should release a burst of neutrinos, and the Mont Blanc team, headed by Carlo Castagnoli, believed that they had discovered such a burst. They were the first to report neutrino detection after other astronomers had reported optical observation of the supernova on 24 February 1987. But four and a half hours after the Mont Blanc burst, which consisted of five events over several seconds, a second burst was recorded independently by the Japanese detector at the Kamioka zinc mine and the US Irvine–Michigan–Brookhaven (IMB) device, located n an Ohio salt mine.

Physicists disagree on what the detection of two distinct signals implies. Because theory predicts only a single neutrino burst from a collapsing star, and because the IMB and Kamioka observations fitted well with theoretical predictions, the general perception among astrophysicists is that the Mont Blanc burst was background noise, most probably caused by penetrating radiation from the surrounding rock. Such a burst would be expected about once every three years simply from random fluctuations.

Saavedra believes it is possible that both the Mont Blanc detection and the later bursts recorded simultaneously in the United States and Japan could have been genuine events linked to SN1987A. This would require the star to have collapsed initially to a neutron star, releasing low-energy neutrinos picked up at Mont Blanc, but below the energy threshold of the IMB and Kamioka devices. A second collapse to a black hole would then explain the neutrino burst recorded by IMB and Kamioka. At Mont Blanc, this burst may have been indistinguishable from the background noise.

Whatever happened, the combination of the Mont Blanc neutrino detector and the Gran Sasso LVD now under construction should give astrophysicists a clearer picture of the next nearby supernova.

Like the Mont Blanc device, the LVD will be a liquid-scintillation detector. The Mont Blanc detector contains 90 tonnes of an organic solvent held in 72 separate 1.5 m^3 tanks. The solvent fluoresces when neutrinos, cosmic muons or other particles pass through it, and sensitive photomultipliers around the tanks detect the emitted light, signalling the passage of the particle. The LVD is planned to be a scaled-up version of the Mont Blanc detector.

Some workers in the field say, however, that the Italians would have been better advised to abandon liquid-scintillation technology for their next detector. With a bank of relatively large scintillation tanks such as those planned for Gran Sasso, it is difficult to track the progress of particles through the detector. On the other hand, the devices at IMB and Kamioka, which work by detecting the Cerenkov radiation given off by particles passing through water, do allow researchers to follow the tracks of particles. This makes it easier to distinguish between different types of particles, and to tell if some events are simply due to faults in the equipment.

Peter Aldhous

OCEANOGRAPHY

Europe in the vanguard?

Turin

Europe should aim to take the lead in oceanographic research in the 1990s, says Umberto Colombo, president of the European Science Foundation (ESF), summing up last week's meeting in Turin on Oceans, Climate and Man, organized by the Foundation San Paolo.

The difficulties are several, including finding funds for oceanographic studies and turning out enough trained scientists, but the rewards would be worth the effort. Given the rapid expansion of oceanographic data and improvements in supercomputing expected over the next decade, many delegates at the Turin meeting argued that it may soon be possible to provide operational forecasts of the behaviour of the oceans, similar to the atmospheric forecasts used in medium-range weather prediction.

Ocean forecasts would provide information useful for shipping, fisheries, pollution control and coastal protection.

Added to this are the spin-offs for climate prediction: the difficulty of including the behaviour of the oceans in general circulation climate models is one of the most serious constraints on the reliable prediction of climate change.

The obvious model for a European ocean forecasting centre is the European Centre for Medium-Range Weather Forecasting (ECMWF), at Reading, England. Since 1979, ECMWF has been providing medium-range (three- to ten-day) weather forecasts for the meteorological agencies of its 18 subscriber countries, and has been a "roaring success", according to John Woods, director of marine and atmospheric sciences at the UK Natural Environment Research Council. But a parallel centre for ocean forecasting is still several years off, he says. The idea is one of many being considered by the European Committee on Ocean and Polar Science, a joint initiative of the European Communities (EC) and the European Science Foundation (ESF), to present to ministers from European governments in 1994.

Nadia Pinardi, from the Italian national research council (CNR) Istituto per lo Studio delle Metodologie Geofisiche Ambientali at Modena, says that there is now enough data collected every 15 days to feed into an ocean forecasting model. The resulting forecasts would be coarser than those produced by meteorologists (ECMWF bases its forecasts on the daily collation of atmospheric data), but would be a useful start, she says.

Pinari also wants the EC's research programmes to provide grants for European scientists to take part in the World Ocean Circulation Experiment (WOCE) — the huge international oceanography project planned to run until 1997 as part of the World Climate Research Programme — and for European researchers to take part in processing the large volume of oceanographic data expected from the European Space Agency's ERS-1 remote-sensing satellite, due for launch next month.

Although the EC's marine science and technology (MAST) programme has been valuable in stimulating European oceanography, Pinadi says, the EC has so far not paid for WOCE research. This is not so much a problem in countries such as Britain and Germany, where there are national programmes of WOCE research. But in most southern European countries, where national governments have shown little interest in WOCE, EC funding is the only hope for oceanographers who wish to participate in the largest research project in their field.

The biggest single obstacle for the progress of oceanography in Europe is undertraining, says Woods. In Britain, for example, only ten per cent of scientists entering the field have previous specialist training in oceanography

Peter Aldhous

HUMAN FRONTIER SCIENCE PROGRAM

A foray into red tape

Tokyo

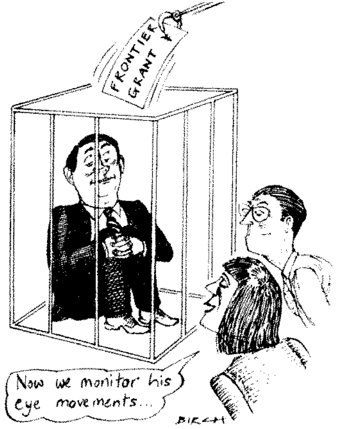
MORE than a hundred scientists from around the world have just won grants under the Human Frontier Science Program (see below), and for all but one of these the hard part is over and all that remains is to spend the money. The lone exception? A Japanese researcher who faces a still unknown number of hurdles before he can put his award to work.

Kenji Kawano of the neuroscience section of the Electrotechnical Laboratory (ETL) in Tsukuba, who will study the neural mechanisms of vision in collaboration with researchers in the Netherlands and the United States, has the dubious privilege of being the first person from a Japanese national laboratory to get a Frontier grant. As such, he will have to blaze a trail through bureaucratic red tape to get his award. Kawano's progress will be watched closely by hundreds of his colleagues in Japan's national laboratories who have hesitated to apply for Frontier grants because of the bureaucratic problems they face if they win an award (see *Nature* **349**, 185; 1991).

The problem confronting Kawano is that at present there is no mechanism for his Frontier grant to enter the accounting system for his institute. Japan's national laboratories, which include all of the country's government-funded research institutes except university-related institutes affiliated to the Ministry of Education, Science and Culture, are all fed from the same general pool of funds. If Kawano's grant is put into this pool, two things will happen. First, the grant will lose all identity and no longer belong to Kawano. And second, because of the rigid ceiling placed on the general account by the Ministry of Finance, funds would have to be displaced and eliminated from elsewhere in the account to make room.

That a Japanese researcher is subject to this Catch-22 is particularly ironic because it is Japan that started the Strasbourg-based Human Frontier Program and Japan still contributes the lion's share of the programme's funds — and these contributions come out of the same general account into which Kawano's award would be funnelled back.

Officials at the Ministry of International Trade and Industry (MITI), to which ETL is affiliated, think they have a solution. They have told Kawano to accept the grant into his own personal bank account. This approach became practical only late last year after the Ministry of Finance introduced special regulations to exempt Frontier grants from personal income tax, as a way to deal with similar bureaucratic problems facing university researchers who won the coveted awards (see *Nature* **347**, 703; 1990).


FRONTIER GRANT
Now we monitor his eye movements...
BIRCH

Under a new set of special regulations just drafted by MITI, Kawano will then be able to buy equipment and supplies with his grant and bring them into his institute — after gaining the official approval of ETL's director by filling in a new set of forms. (Researchers were not previously allowed to bring such 'personal' items into the laboratory.) When Kawano no longer has a use for the equipment, he can 'donate' it to ETL by filling in yet another set of forms.

Kawano says he is reasonably happy with this proposal but is apprehensive about how it is all going to work. One concern is that he needs someone to feed the monkeys for his experiments on the neural mechanisms of eye movement. But under MITI's proposal he cannot employ a part-time assistant with his Frontier grant because an assistant does not qualify as 'equipment' or 'supplies'. However, Masayoshi Hamada of MITI's Agency of Industrial Science and Technology says that Kawano can get around this problem by employing a student under an existing system for inviting 'visiting scientists' to ETL. All Kawano has to do is claim that the monkey feeder is a 'capable researcher' who deserves to be invited into the institute.

MITI officials are clearly on Kawano's side and will do everything possible to smooth his journey through the unknown bureaucratic jungle that awaits him. And in the end, he may end up better off than researchers who receive equal-sized grants from other sources.

Because the money will be deposited in his own personal bank account under his own management, Kawano will not have to pay ETL any overhead from his grant. Normally, researchers at ETL and other national laboratories have about ten per cent automatically lopped off their grants to cover such things as gas and electricity bills and administrative costs.

Kawano should also be able to earn several thousand dollars in interest on his grant while it sits in the bank. MITI officials say it is entirely up to him what he does with the extra money. After all, with his extra duties as trail-blazer and guinea pig, he will have earned it. **David Swinbanks**

US leads race for Human Frontier grants

Tokyo

JAPAN pays most of the bill, but it is US researchers who come off best in the allocation of grants for international research on the brain and molecular biology announced last week by the Human Frontier Science Program Organization (HFSPO) in Strasbourg.

US scientists lead 15 out of the 32 successful international research teams, which will get grants ranging in value from about $500,000 to $900,000 for three years. And 46 out of the 139 scientists in the teams are from the United States, far more than from any of the other participating countries.

The dominance of the United States shows the growing interest of US scientists in the Japanese-inspired programme. Eighty out of the 239 principal applicants for grants were from the United States, more than twice the number from any other nation.

HFPSO was set up under Japan's initiative by the Western summit nations (Canada, France, Italy, Japan, the United Kingdom, the United States and Germany) and the European Communities in 1989. Switzerland also recently became a member. But Japan still provides more than 75% of the annual budget of about $26 million for the programme.

The United States, on the other hand, has made no solid commitment to contribute money to the programme, although an official of the National Institutes of Health (NIH) recently wrote a letter to Sir James Gowans, the British secretary-general of HFPSO, promising 'in kind' support (see *Nature* **350**, 97; 1991); this support would apparently add up to a few hundred thousand dollars a year, spent in the form of salaries paid to Frontier fellowship winners who go to institutes affiliated with NIH. US government officials argue that the United States already does a tremendous amount to support international research on the brain and molecular biology through NIH and other US-funded grant programmes and so does not need to contribute much to Frontiers.

Japan fared reasonably well compared with the other non-US member countries. Japanese scientists lead three of the successful teams and there are 27 Japanese among the grant recipients. But the number of Japanese principal grant applicants was greatly reduced this year — to 29, compared with 55 last year. The fall-off in applicants is apparently due in part to fear of bureaucratic barriers that face successful applicants in Japan. **D.S.**

UNIVERSITY-INDUSTRY COLLABORATION

Questions on drug pact

Boston

THE Harvard-affiliated Dana-Farber Cancer nstitute has signed a cooperative research agreement with a leading pharmaceutical company that is raising anew questions about just how closely a university should collaborate with commercial interests.

In the arrangement, announced last month, Dana-Farber will receive up to $100 million over the next ten years from Sandoz Pharmaceuticals Ltd, the US subsidiary of he Swiss company. The two will cooperate to develop anticancer drugs based on increased knowledge of cell signal transduction. Many researchers in the field believe that they now understand cellular signalling processes well enough to begin trying in earnest to develop drugs that will target specific signal transduction defects — problems in cells' chemical signals that give rise to the unregulated growth of cancer cells.

The agreement, due to begin on 1 January 1992, marks one of the largest investments so far by a pharmaceutical company in an academic research and teaching institution. But perhaps the most noteworthy feature of the plan is the way it is designed to pair academic researchers and corporate chemists in what David Livingston, vice president of Dana-Farber calls a "joint discovery" process.

The Dana-Farber Institute will devote roughly 10 per cent of the work time of its scientific staff to Sandoz projects. In addition, the arrangement calls for about 100 Sandoz chemists to work at the Dana-Farber side by side with its more than 400 researchers and postdoctoral fellows. Finally, in a departure for Harvard and other universities involved in large academic–corporate arrangements, patent rights and royalties from discoveries in the new programme will be shared by the two institutions rather than held by the academic institution and licensed to the company.

Livingston says he believes that the applied nature of the collaboration will fit easily within Dana-Farber's mission, because development of cancer treatment "is part of our mandate as a cancer center". But some critics worry that the arrangement could eventually weaken the institute's commitment to basic research. And Richard Kolodner, who conducts basic research at the Dana-Farber and chairs its division of cellular and molecular biology, says that many "legitimate questions" exist "about how something like this might influence hiring, or shift our mission."

As Kolodner observes, the Sandoz arrangement is unabashedly "goal-oriented", a characteristic that he said at least semantically separates it from previous collaborative plans involving Harvard. "Anyone here who wants some of this money will have to look at how their research might contribute to the development of drugs," Kolodner notes.

Such a feature could skew the direction of the institute's research, he says, but "it could also stimulate people to think about their research in new ways." All in all, Kolodner remains sanguine. "If you're at a cancer center, you can't help but think about the central problem at hand — treating and curing the disease."

Seth Shulman

PHARMACEUTICAL PATENTS

EC row looms over patents

London

A EUROPEAN Commission proposal to extend the life of patents on new drugs to a maximum of 16 years after a drug's commercial launch (see *Nature* 344, 482; 1990) has been rejected by the British government, to the annoyance of UK pharmaceutical companies. The British rejection of the plan, which aims to bring European drug patenting in line with US and Japanese practice, means that a row is likely when ministers from European Communities (EC) member states meet to discuss the proposal later this year — France and Italy have already passed their own laws based on the Commission's ideas.

The problem facing European pharmaceutical companies is that drugs must be patented before clinical trials begin. By the time a drug is licensed for commercial use, a 20-year patent often has only eight years left to run. After this time, cheaper 'generic' copies of the drug can be sold, made by companies that have not had to shoulder the burden of high research and development costs.

The UK government says it will support the extension of drug patents, but only up to 13 years from a drug's launch. (The maximum effective length of patent protection in the United States is now 14 years, and in Japan 15 years.) The Commission's plan goes further than is necessary to help European companies compete with their US and Japanese competitors, said industry minister Lord Hesketh to the House of Lords, and the effect would be to discriminate against generic companies, and increase the costs of drugs.

However, the Association of the British Pharmaceutical Industry, which includes generic manufacturers among its membership, opposes the government's stand.

Apart from Britain, most of the other EC states with large pharmaceutical industries are in agreement with the Commission's plan, although the less-industrialized countries of southern Europe are expected to oppose the extension.

Peter Aldhous

DEMOGRAPHY

Census sex puzzle

New Delhi

INDIA added another 160 million people in the past ten years to bring its population to 844 million. According to the 1991 census, the growth rate decreased by only 1.2 per cent compared to the previous decade.

Demographers are puzzled because the ratio of females to males in the population has declined, following an upward swing measured in the 1981 census. There are now 929 females for every 1,000 males, as compared with 934 in 1981.

At the turn of the century, India had 972 females for every 1,000 males, but the ratio had been steadily falling until the 1981 census. The reasons for the uneven sex ratio included a preference for male children, resulting in neglect of female babies, as well as a relative gap in health conditions between the two sexes — factors that many hoped had started to change.

The fact that the female/male ratio has fallen back since 1981 despite numerous women-centred health and nutrition programmes has set off a debate. Feminist groups believe that one cause is the selective abortion of females after sex selection tests offered by private clinics, which are mushrooming in cities and even small towns. Demographers, however, say the practice is not so widespread as to affect the sex ratio, and have called for an in-depth study to look for other reasons.

K. S. Jayaraman

SOUTH AFRICA

Saving the shark

Johannesburg

SOUTH Africa is to become the first nation to give official protection to the great white shark, *Carcharodon carcharias*. The Minister of Environment Affairs, Louis Pienaar, announced on 11 April that regulations to protect the species in South African waters will be promulgated shortly.

The legislation was necessary, Pienaar said, because international trophy hunters are starting to focus their attention on the South African coast. The shark's jaws command up to R12,000 as trophies, and populations of the predator have been decimated in some parts of the world.

In addition to making it illegal to catch great whites without permits, the legislation will forbid the sale of the species.

In future, permits will be issued only for research purposes or to the Natal Sharks Board, which is responsible for catching approximately 50 juveniles of this species each year in the shark nets that protect bathing beaches. The effect of the conservation measures on the local great white population will be monitored by Dr Len Compagno of the Shark Research Centre at the South African Museum, who is conducting research on its ecology.

Michael Cherry

REVIEW SYSTEMS

Computerized grants project is unveiled

Bethesda, Maryland

The halls of the National Institutes of Health (NIH) are paved with grant applications. So are the offices. Come deadline day, when 35,000 applications come in at the last minute, the Federal Express trucks are gridlocked in the parking lot unloading funding appeals. By the time NIH finish making 75 copies of each application and distributing them to all the appropriate offices, the agency is buried under more than 2.5 million applications, each about a hundred pages long — over a thousand tonnes of paper.

In the electronic age, this flood of paper is an embarrassing thorn in the side of the NIH and other US science agencies. Years of vague proposals to computerize the process have produced some abortive trials, several committees and plenty of excuses.

But now, a handful of computer evangelists with some hot machines are finally making progress towards the grail of grant-making: the all-electronic application and review. No paper, no post — nothing but bits and bytes.

Earlier this month, grants officials unveiled their plans for an electronic future at a meeting of the Division of Research Grants advisory board attended by senior NIH staff. Experimental grant software is nearing completion, they said, and will be tested in preliminary trials in early fall at the University of South Carolina, the University of Washington and another, yet unnamed, university.

In addition, a collaboration with the National Science Foundation (NSF) is aiming at a common applications form and software that could be used at both science agencies. The two combined receive some 70 per cent of all grant applications to the federal government.

Researchers are making progress towards the grail of grant-making: the all-electronic application and review. No paper, no post — nothing but bits and bytes.

John Mathis is the motive force behind the most ambitious of the NIH projects — one that will eventually not only allow scientists to create and submit their proposals electronically, but will also let NIH computers read and categorize them without human help. Mathis's vision of a computerized grant-making machine is a mix of simple personal-computer software and complex artificial-intelligence (AI) programs.

Known as the EGAD (Electronic Application and Grant) project, the effort is about to show its first face — a software program for distribution to universities that will check applications for completeness and accuracy in real time as a researcher enters the information.

The software (designed for Macintosh or IBM-compatible computers) will make sure that the numbers add up at the bottom of the financial portion, and that the researcher has not accidentally given something like 'biology department' as his or her last name.

Typing time and labour are just the most obvious of the savings. Mathis hopes that a sophisticated AI program that is still under design will also take over the tedious chore of assigning applications to the appropriate NIH institute and study section, a task that now requires a panel of in-house scientists.

The AI program, known as 'the referral assistant', will scan an application, looking for information that reveals its scientific field and subject. Several techniques for doing this are being considered, including keyword searches and citation analysis, which would use the references cited at the end of the application as a guide to the science within.

At the National Science Foundation, however, progress on an electronic grants package has been tempered by a sobering example of how hard the transition can be.

In 1988, NSF started a project known as EXPRES to produce a program that would allow scientists to create and submit grant data electronically. Two universities, Carnegie Mellon and the University of Michigan, were to develop a standard 'document

Electronic review: mixed messages

Washington

Science agencies may save a lot of paper-shuffling by receiving grant applications electronically, but the real delay and expense is still the review process after the applications arrive. Both the National Institutes of Health (NIH) and the National Science Foundation (NSF) fly hundreds of researchers to Washington each year to staff panels that review stacks of applications, ranking them in order of scientific interest. The entire review process costs the two agencies some $80 million each year, and can take up to nine months to complete.

Both agencies are considering replacing the traditional face-to-face review group with their electronic equivalents. But as a few trial projects have shown, getting scientists to give their full attention to peer review while sitting in their own offices is a challenge that may defeat even computers.

In an experiment conducted last year, NSF submitted 52 applications to electronic peer-review. After the agency mailed the applications by regular post, the panellists submitted their reviews and exchanged comments over Internet, a nationwide computer network.

Overall, the experiment was generally a success. NSF found the comments of the 'e-panels' to be more thoughtful and the reviewers more prepared than in similar face-to-face panels. But they also noted that interaction among the panel members ranged from minimal to virtually non-existent.

"There was very little exchange of e-mail," one officer wrote in a NSF report on the project. "In one panel it was because the opinions were unanimous. The other panel was a disaster. I couldn't get them to do much of anything."

Reviewers, of course, liked not having to travel to Washington, DC. (So did NSF. Bringing a scientist to headquarters costs the agency about $1,000 per day. NIH spend more than $20,000 for each of their three-day study sections.) Most reviewers also said they could live without the body language and verbal clues of a face-to-face panel. For small (3-4 people) groups with three to six proposals each, the extra time to prepare thoughtful comments and opinions was worth the lost personal interactions, they said.

NSF, which tends to have smaller review groups and to give programme officers more say over funding decisions, may eventually be able to adopt e-mail reviews for the two-thirds of all reviews that are now conducted at least partly with face-to-face groups.

The NIH, on the other hand, depend almost exclusively on their study sections, which usually number about 20 people — probably too large for e-mail review. For some special reviews, however, NIH assemble panels of as few as five reviewers. In past experiments, NIH have occasionally convened panels by conference call and — in at least one abortive trial — mail.

The agency found that conference calls appear to work relatively well at a pinch, although they have none of the advantages of face-to-face reviews (the nonverbal communications) or e-mail reviews (the more careful consideration). But getting reviewers to respond by mail turned out to be hopeless. Almost all the comments came in late, and some never came in at all. "We had to throw some reviews out altogether because we didn't get even three responses," says John Mathis, who ran the NIH experiment.

Until researchers learn to type as fast as they can talk, they are likely to resist being handed a stack of grants for electronic review. Although the trend towards increased use of e-mail is clear, for now, most reviewers would rather fly to Washington than write a ream of comments about somebody else's grant. **C.A.**

architecture' or text format, as well as two different word processor programs that could understand the format. But the universities never reached the point at which their software could share files, and the project was killed last year.

The only concrete product to come from the NSF project was a simple text processor called PS-Express, which is now widely distributed among scientists. It converts a simple list of data elements (such as 'Last Name = Mathis') into a file written in the common Postscript graphics language. When sent to a Postscript-compatible printer, the file prints out an entire NSF application form with all data filled in.

NSF also accepts PS-Express files electronically, over the Internet computer network. Some 200 applications have been filed that way in the past two years.

But electronic submission at NSF "is still a very hand-held process," says Lawrence Edwards, NSF senior project manager for office information systems. "When we get an application electronically, I print it out, take it downstairs and put it in the queue, just like it came in the mail."

NSF is now developing a simple 'extract' program that will read the PS-Express files and automatically transfer 20–30 essential data elements into the main NSF database, without the need to type it in. That saves time, and more importantly, errors.

But more ambitious projects are in abeyance until the NIH experiments prove that researchers, administrators, agency officials and the technology are all ready for the leap.

So far, government acceptance of the electronic grant projects has been cautious — for good reason. Although the technology has come far in the past ten years, most computer monitors are still barely adequate to display an entire application page at once, clearly or not. "I'd hate to read a proposal on the screen," Edwards says.

And at NIH, Mathis is still fighting computer phobia. "We're waiting for a generational change," he says. "People keep telling me, "John, what you're doing is terrific. I just hope I retire first."

Mathis takes the long view. "Everybody knows that (electronic grants) are inevitable," he says, "it's just a question of when to buy in. If you want to be on the cutting edge and to have a lot of fun, you do it now. Otherwise, you step back and let everybody work the bugs out."

Bugs or not, cultural resistance remains the largest hurdle to electronic grants. Mathis and his staff spend much of their time visiting universities, other parts of NIH and federal agencies to preach the gospel of computerized applications. These "dog and pony shows", as Mathis describes them, are intended to create a groundswell of demand. Until Mathis and Edwards can convince university administrators that electronic grants save time and trouble, the weak link in the chain will continue to be the human one.

Christopher Anderson

TELECOMMUNICATIONS

Satellite aids sore fingers

Munich

As anyone who has tried in recent months to place a call from western Germany to eastern Germany can attest, the two telephone systems are anything but unified. A re-dial button or a rubber finger is a virtual necessity, since the few available lines are always busy.

But now the national telephone company Telekom is bringing in high technology to widen the bottleneck in at least a few geographic areas until a more thorough upgrading of eastern Germany's antiquated system can be carried out. Earlier this month, Telekom began switching calls between some eastern and western cities by satellite in an efficient, if expensive, attempt to alleviate the situation.

From 5 April, Telekom made available 30 additional satellite lines between the western city of Hamburg and the eastern city of Dresden to complement the paltry 24 land lines previously available.

Hamburg has a population of more than 1.6 million; Dresden has 500,000 residents and is the third-largest city in eastern Germany. An additional 36 lines are expected to be added later this month.

It is the first time a satellite has been used to route public calls between the two parts of Germany. The satellite, known as Kopernikus, is normally used for intercontinental calls.

But the stopgap use of the satellite does little to ease the misery of private and corporate customers in other regions of Germany. Telekom, and the Ministry of Post and Telecommunications to which it belongs, have been lambasted in the German press for their sluggish response to the immediate need for more telephone lines between the two regions of Germany. As Telekom has a monopoly on offering basic telephone service, there is nowhere else the customers can turn.

Telekom is planning to invest DM55,000 million (about $33,000) by 1997 to rebuild the neglected eastern German telephone infrastructure. A temporary network, known as an overlay network, is expected to be in place by July. It will be used as an adjunct to the existing network in eastern Germany, most of which was installed back in the 1920s.

Steven Dickman

MONEY MATTERS

Gaussian curve graces banknote

Göttingen

The German mathematician and astronomer Carl Friedrich Gauss (1777–1855) is honoured on a new German 10-mark banknote issued on 16 April in Göttingen. The bill marks the second time in as many years that a German banknote has been issued featuring a scientific luminary. Last year, immunologist Paul Ehrlich was so honoured (see *Nature* 347, 415; 1990).

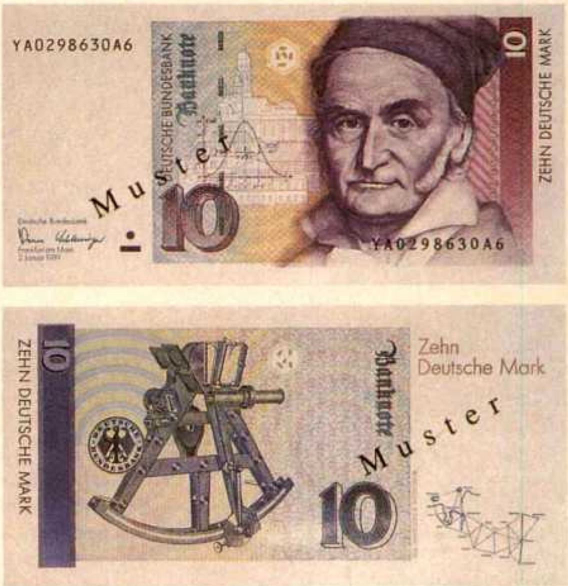
Gauss, for whom approximately 50 mathematical laws, formulae and methods have been named, was head of the Göttingen astronomical observatory from 1807 to 1855. One of the founders of modern geophysics, Gauss became famous during his lifetime for his method of measuring magnetic fields in absolute units, which later became known as gaussian units, including the gauss (for magnetic field strength).

In addition to a portrait of Gauss, the face of the note features the familiar gaussian distribution. The reverse side features a 'viceheliotrope', a type of sextant used for surveying invented by Gauss to divert a ray of sunlight to a distant observer. Circles in the background represent stylized planetary orbits and magnetic fields.

Gauss is latest in the line of scientists to receive recognition on German currency.

In 1992, a third figure from German scientific history, this time a woman, will appear on a new 500-mark note: Maria Sibylla Merian (1647–1717), who compiled a famous catalogue of drawings of insects. The series of notes is designed to depict great personalities of German cultural life.

Steven Dickman



Gauss is latest in the line of scientists to receive recognition on German currency.

Two views of the new Germany

SIR — The comments of Frits L. Meijler (*Nature* **350**, 268; 1991) on the purported German character are hurtful in two ways: (1) It is horrible to see how deeply he has been hurt, and still feels; (2) I resent his presenting a harsh verdict, then acknowledging that E. Guthy (*Nature* **348**, 670; 1990) did his best but claiming that Guthy did not have a right to say what he did because he is German. In effect, the accused is not allowed to defend himself because he is being accused.

Meijler is a generation older than I am, judging from his letter. For me, things do look different. It is my firm belief that, if there is a common strain among Germans born after 1950, it is a constant if unexpressed awareness of Auschwitz. It has pervaded the political thinking of my generation to such an extent that many foreigners consider its effects rather strange. A friend of mine once remarked, "What Germans cannot understand is that I can say, I am an American, and I just don't feel bad about it". I learned only when I went to the United States that other peoples do not have a taboo on national feelings. Germans do, even after 9 November 1989, because 9 November 1938 is by no means forgotten. If there are some groups of thoughtless people, such as those who threw stones at Poles when the border was opened for them this week, they are a reason for concern, like the soccer hooligans in Great Britain; but if there is anything typical in the incident, it is the immediate sense of alarm in the public reaction.

If Meijler related his personal feelings on that matter, may I relate mine? How it is to grow up, becoming slowly aware of a horrible past, learning that there is a cause of great shame although I had not done anything wrong? Knowing that my father fought in Hitler's army, from loyalty to his country, as is today considered normal in any other country but Germany? I left Europe, in part to leave all this behind. To no avail.

Around the age of 15, I was told that one can tell a Jew by the look on his face. I was outraged, thought this to be a racist opinion. Then in Califormia I met a very beautiful young woman with a face unlike any I had ever seen before. One day I saw in a bookstore a book of photographs, apparently taken by an SS officer at the ramps in Auschwitz. There were long columns of people, bearded men with hats, then a close-up of the first row of the women — and there was my friend's face, six times, with the knowledge of death in their eyes.

Nothing reveals the backward direction of Meijler's view more than the use of the phrase "Grossdeutschland". All right, Germany won the soccer championship last year, so what? No peoples are more eager to get along with their neighbours than Germany, precisely because of what happened to Meijler's parents. Regaining a sense of national feeling is probably a healthy process. When the borders opened on that day in November, I felt that an incredible burden had been taken from me which I had been carrying for so long that I did not feel it any more. I was utterly surprised at myself. What I observe now among the public is not an exaggerated sense of pride, but rather a sense of being 'all right' again.

A more genuine reason for concern now perhaps is that the former East Germans are not used to dealing with people from other nations. They definitely react in a different way from Westerners to Turks, Yugoslavs and Poles. They will have to learn.

FALK KOENEMANN

Institut für Mineralogie, RWTH,
D-5100 Aachen, Germany

SIR — I read with interest the striking article on "Germany in perspective" by E. Guthy about a revival of prejudice against Germans based now on German "national or racial characteristics". It is surprising but true that at this stage of development in genetics such articles are not only desirable but indeed very much needed. Even among scientists there are still some who tend to forget that neither a German nor for that matter a Jewish gene was ever sequenced.

During the pre-Nazi and Nazi period, the most famous champions of the Nazi 'genetics' were the German university professors E. Fischer and Othmar von Verschuer. They accepted as a dogma the superior 'national and racial' characteristics of Germans and taught that the social, cultural and political activities of some religious and/or ethnic minorities are genetically predetermined and therefore forced sterilization or 'elimination' (that is, robbery and murder) of certain minorities is justified. Now, apparently, some try to explain German politics in the same but reverse vein by assuming the existence of a malicious 'German gene'. It would appear that the Fischer–Verschuer obscurantism in genetics (and that of their ambitious postdoc Josef Mengele) is still alive and kicking even among those with a scientific background. After all, the very same von Verschuer remained for almost 20 years an active university professor of 'anthropology' and genetics in post-war Germany (cf. Benno Müller-Hill *The Murderous Science*, Oxford University Press, 1989). For such reasons Guthy's wise words should be doubly appreciated.

On the other hand, I share the concerns of W. Frank Harris and of J. F. MacKenzie (*Nature* **347**, 510; 1990) that, with the new economic power and without the constraints of partition, the destructive and inhuman forces of the past might loom up in the post-Bittburg Germany, although I would not look for their origins in a 'German DNA'. It is true, for instance, that Chancellor Helmut Kohl organized a wreath-laying ceremony on SS graves in Bittburg in the 1980s, but this was not genetically predetermined.

Many other frightening academic and political phenomena (as the Verschuer and Bittburg cases) were tolerated during the four-and-a-half decades of post-war developments in Germany. One can therefore understand the concerns (mentioned in your leading article in *Nature* **346**, 203; 1990) of Mrs Margaret Thatcher and the group of academic historians, who "wondered whether some of the unhappy (German) characteristics of the past might reemerge in 10 or 20 years with just as destructive consequences". It is not irrelevant in this connection that the facilities for manufacture of poisonous gas in Libya, Iraq and Syria were provided by (among others) contemporary German technology and business, albeit run by indigenous talent. I do not accept the theory that this could have been done while the German authorities were sleeping. Neither do I believe that a 'German DNA' is responsible.

The differences between Guthy's and Mrs Thatcher's perspectives on Germany arise from a different approach to the problem. Guthy in his article covers centuries, whereas Mrs Thatcher is influenced by living memory. I gladly agree with Guthy that the past centuries of glorious German history are a guarantee for the future glorious centuries. The question remains of what is going to happen in the next 10 to 20 years. There are many good reasons for optimism as well as for anxiety and vigilance.

RUDOLF VRBA

University of British Columbia,
Department of Pharmacology &
Therapeutics,
Faculty of Medicine,
2176 Health Sciences Mall,
Vancouver, BC, Canada V6T 1W5

Mix-up

SIR — I read with interest the book review by Joseph F. Sambrook about genomic genomes (*Nature* **349**, 752; 1991). My father, Lionel Penrose (1898–1972), geneticist and friend of Frances Partridge, might well have felt flattered to be taken for Sir Francis Galton (1822–1911), who was a founder of human genetics.

It was only in 1956 that the number of human chromosomes was clearly shown to be 23 pairs, and the chromosomal abnormality in Down's syndrome was found to be trisomy 21 in 1959.

SHIRLEY HODGSON

Guy's Hospital,
Division of Medical and Molecular
Genetics,
7th & 8th Floors, Guy's Tower,
St Thomas Street, London SE1 9RT, UK

Correction

In the letter entitled "Early case of AIDS in the USA" (*Nature* **347**, 509; 1990), the authors should have been listed as both R. F. Garry (Tulane University) and M. H. Witte (University of Arizona). □

Does climate still matter?

Jesse H. Ausubel

We may be discovering climate as it becomes less important to well-being. A range of technologies appears to have lessened the vulnerability of human societies to climate variation.

Amidst widespread agreement that the planet is committed to at least some climatic change induced by human activity, there is growing pressure to "slow the greenhouse express" (ref. 1). Here I examine climate through the lens of technology and innovation, to clarify what adaptations have succeeded and the trends in vulnerability to climate. I also examine whether the greenhouse effect by itself will call into play new technologies, or whether the evolution of technology will largely be 'business as usual' regardless of climate change. Finally, I identify some ways that government may assist adaptation.

My focus is on adaptability of human systems, including agriculture. Adaptability of ecosystems and the ethics of human behaviour that brings about large-scale transformations of the Earth must also be considered in balancing responses to the greenhouse issue.

What are often labelled adaptive measures are themselves the impacts of climatic change. Innumerable adaptations in food, clothing and shelter are responses to the spatial and temporal variability of climate. Humans do not wait guilelessly to receive an impact, bear the loss, then respond with an adaptation. Rather they attempt to anticipate and forstall problems.

Innovations

Technical innovations relevant to climate and their diffusion occur in all societies and sectors and in many forms. Technology includes both hardware (for example, materials, physical structures, devices and machines) and software (rules and recipes for behaviour).

Illustrative innovations of hardware are cisterns and dams to store water, tractors to speed rapid harvests, and new crop cultivars to reduce susceptibility to drought. Perhaps less obvious, but of great importance for adaptation to climate, are information technologies. In the United States, during several years in the 1980s sales of information technologies to the agricultural sector were comparable to sales of farm equipment. The long history of software innovations includes tide tables, irrigation scheduling, and weather forecasts. Along with the readily classified hardware and software are climate-related behavioural, social and institutional innovations, such as agricultural credit banks, national parks, green political parties and flood insurance.

Software and social innovations are almost always indispensable for the technology of new hardware. Major innovations, in transportation for example, are in fact clusters of innovations involving not only new materials and physical processes but also new forms of social organization, including financing and management. Transportation systems exemplify technology that has been important in adaptation to climate through expanding the availability of food from a local to a global scale. Reliance on food from afar not only diversifies diet, but also spreads production risks across more climatic zones.

Early communities drew the bulk of their food from small areas. One of the earliest city states, Uruk in Mesopotamia, probably grew most of its sustenance except for animals within 20 kilometres of the city walls[2]. Two millennia later, Greece and Rome obtained most of their food from overseas colonies across the Aegean and Mediterranean seas[3]. At its height, Rome acquired 200,000 tons of grain annually for its one million inhabitants, most of it shipped from Africa, Sardinia and Sicily. What marine transportation did in the classical world, the steam locomotive did in the nineteenth century, halving the cost of land transport. The railroads penetrated the great land masses of North America and Australasia. Their operations were little affected by climate, topography or other local conditions. Great parts of the new worlds were dedicated to cultivation of single crops to supply world markets and to smooth availability through the year.

Technological inventions and innovations that have had roles in human ability to adapt to climate over the last 100 years or so range widely[4]: food preservatives (1873) to overcome problems of seasonal food production; light bulbs (1879) to make work safe and effective indoors; aluminum (1887) and other structural materials to resist environmental deterioration; refrigeration (1895) and air-conditioning (1902, 1906) to facilitate activity in hot seasons and locations; automobiles (1890s) to provide personal transportation that is much less sensitive to climate than horses or pedal bicycles; mechanical windshield wipers (1916) to see in the rain; antifreeze (ethylene glycol) (1929) to safeguard motors in winter; frozen food (first sales, 1930) to diversify diet among regions and seasons; radio-beam navigation (1934) to fly in poor visibility; and weather (1960) and Earth resources (1972) satellites for analysis and forecasts of weather and climate.

In many respects we seem to be 'climate-proofing' society, making ourselves less subject to natural phenomena. For centuries and millennia we relied mainly on behavioural and social adaptation. We took siestas when the sun was high and sought refuge in hill stations in the monsoon season. Large pastoral and nomadic populations followed the seasonal availability of resources and avoided climatic stresses. Much of the planet remained seasonally or entirely uninhabitable for climatic reasons. With current technology many people can live in virtually any climate that now exists. Modern water supply and heating and ventilation systems, along with medicines (for example, quinine and vaccines) and public health measures, have enabled large populations to inhabit formerly uninhabitable regions. By 1980 the population in semiarid, desert and mountain regions had passed 35 million or 15 per cent of the US population[5]. Lacking modern technology, these zones accomodated less than one per cent of the population in 1860 and six per cent in 1920.

Preferences

The ability to colonize almost the entire planet is due to the human ability to carry with us that particular range of environment in which we can survive and prosper[6]. In wealthier societies, preferences are shifting toward hot and dry climates that were forbidding a century ago. Evidence of lessening human vulnerability is also found in health. For example, a flattening of monthly rates of total mortality in Japan between 1899 and 1973 is explained partly by diminution of the previous, climatically driven seasonal peaks[7].

Production can now proceed more or less continuously in severe environments. For example, North Sea offshore oil platforms operate 24 hours per day, 365 days per year. At a price, services, such as aviation, are now available at almost all times and places. Aviation began as a system that was extremely sensitive to weather and is increasingly less so, due to expanded range; avionics, radar and guidance systems; understanding of thunderstorms, wind shear, and other weather phenomena; and changes in construction materials. Crops and livestock can now be produced 'indoors' protected from the elements. In some cases, alternatives to outdoor production are so advantageous that a crop is displaced. Originally spurred by the need for supplies in wartime, synthetic rubber from petrochemical feedstocks, which is not subject to the vagaries of pests, droughts and floods, and other risks outdoors, has overwhelmed natural rubber from trees. In 1990, ten per cent of US fish production was in the controlled environment of fish farms[8], up from one per cent in 1980 and projected to reach 20 per cent in 2000.

Consumption is also insulating itself from environment. Inside most shopping malls, for example, only fashions or decorations signal the season. Sports are increasingly played in domed stadiums isolated from the weather. In affluent societies, winter vacations in warm climates have become a popular adaptation to escape climatic impacts.

Forecasting is itself a key technology that reduces vulnerability to weather and hence climate. Forecasts can help accommodate peak loads in electric power systems during heat waves. Improved forecasting, in conjunction with increased incomes and better transportation, has also enabled more people to enjoy recreation in all seasons.

Synchronicity

The decline of 'synchronicity', the naturally enforced time regimen of social groups, is a feature of life in advanced societies. In agricultural societies, the rhythm of life is strongly determined and coordinated for almost all by the seasons and the associated demands for labour in the field. In advanced economies, where both production and consumption may proceed almost continously and only about five per cent of the population farms, weather and climate no longer control schedules. The fact that the peak season for holidays in advanced societies is the late summer, a peak season for labour in agricultural societies, indicates the transformation that has taken place.

In the late 1970s and early 1980s a group of US researchers explored the 'lessening' hypothesis of climate impacts[9], which states that persistent and adaptive societies, through their technological and social organization, lessen the impacts of recurrent climatic fluctuations of similar magnitude upon the directly susceptible population and indirectly lessen the impacts on the entire society. In the cases studied, substantial evidence was found to support the hypothesis of lessening impacts. For example, in the US Great Plains, the most severe disruptions to livelihood and health occurred during the earliest periods, when incidences of malnutrition and starvation were recorded. Investigation of the more recent periods showed much smaller impacts for comparable drought stress, because of a variety of adjustments and strategies, including more extensive and effective anticipatory action.

An alternative to the lessening hypothesis is that increasingly elaborate technical and social systems insulate us from the adverse effects of recurrent climatic fluctuation at the cost of increased vulnerability to catastrophe from less frequent natural and social perturbations. In a global economy, such vulnerability might be devolved or shared ever more widely. Presumably this vulnerability to catastrophe, surprise or nonlinear effects is what worries many about the greenhouse effect. But the evidence seems to weigh against the suggestion that technology, lifestyles and other forces are creating a more 'brittle' system in the face of climate change.

Society appears to proceed along 'technological trajectories' that enable, for example, more travel, more financial transactions, and more messages. The succession of technologies that make possible this increased activity appears to diminish in sensitivity to climate. Although it was usually true that neither rain nor sleet stayed the American postman from his rounds, no system of postmen could be as faithful as the modern telecommunications system that now carries a much larger share of messages than the old system of letters. Similarly, a system of energy from wood and hay was more climatically sensitive than one reliant on oil and natural gas. Water and wind power are, of course, more sensitive to climate. In the late eleventh century, the areas under Norman rule in England had about one water mill for every 50 households[10], providing power to grind grains, work metal, weave textiles and cut wood. In 1694 France had 80,000 flour mills, 15,000 industrial mills, and 500 iron and metallurgical works, altogether almost 100,000 facilities powered by wind and water. Although such an industrial infrastructure is tightly adapted using climatic resources, it is also vulnerable to climate variability and change.

The trend toward less climatic vulnerability also exists in transportation. Well into the nineteenth century, sailing vessels were the preferred long-distance transport and frequently becalmed. World steamship tonnage exceeded sailing tonnage only in 1893. Although coal cost more than wind, steamships rapidly became cheaper as well as faster than sailing ships, because their schedules were more regular and avoided the circuitous routes required by sailing vessels. Transport underground through tunnels by high-speed magnetically levitated trains is already on the drawing boards in Japan; such systems would be less sensitive to climate than the surface and air systems now in use.

Climate is only one of several factors that have driven the evolution of systems of communications, transport and energy. It is probably secondary. But vulnerability to climate and other environmental forces may be a good proxy for quality and reliability of service. To the extent that the systems evolve in the direction of higher quality and reliability, these trajectories of development may also decrease vulnerability of major systems to climatic change. It would be useful to identify exceptions to this pattern, should they exist.

As incomes depend less on activities out of doors, societies become less vulnerable to climate. The trend in all developed countries since the industrial revolution began is away from employment outdoors (Fig. 1) and toward employment in the service sector, most of which is in climate-controlled office buildings and shops. With a lag of 50–100 years the same trend is found in less developed countries, where 40–50 per cent of the population is now engaged in agriculture. If the current trend continues, this fraction should diminish to 20–25 per cent by 2050.

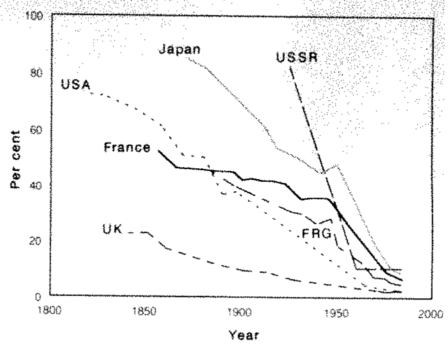


FIG. 1 Share of the workforce employed in agriculture[21].

What are the times characteristic of technical innovation and diffusion of technologies in relation to the time of human-induced climatic change? Taking account of both CO_2 and non-CO_2 greenhouse gases, major climatic shifts are expected during 40–50 years (ref. 11). A retrospect on technology during the past century suggests the extent of change during the decades to come: in 1890 there was little farming in California and Australia, and key technologies did not yet exist or were not widely applied to impound and transport water, or to transport and store agricultural products promptly; in 1903 the Wright Brothers flew 59 seconds at Kitty Hawk, North Carolina, whereas in 1990 in the United States, over 500 million air passengers flew around 450 × 10^9 miles[12]; commercial transatlantic aviation, relying on the jet engine, superseded travel by ocean liners about 1960; tourism has been extended to Antarctica, and scientific bases there are occupied year round, despite the fact that it was only in 1911–1912 that Roald Amundsen reached the South Pole and Robert Scott died there; penicillin, the first important antibiotic, was discovered by Alexander Fleming in 1928, and large-scale production began only as recently as 1943; nuclear power for electricity generation first came into use in the late 1950s, but within about 30 years it was able to provide over 70 per cent of France's electric power; until 1965 no satellites were used for any routine application, whereas satellite systems now girdle the Earth, watching for storms, relaying communications, and helping ships and planes navigate anywhere on the globe; and finally, although the microprocessor was only introduced in 1971 and the personal computer appeared about 1977, Americans are now using over 50 million personal computers.

During the period in question and with or without climatic change, technology will transform the way people live. Food, energy, transportation and all the other systems that support human life and the economy will be changed by technologies that can be glimpsed now, such as genetic engineering, fusion, superconductivity and desalination, as well as by technologies yet to be easily pictured. Fifty to a hundred years will allow the replacement of most major technolgical systems. Indeed, 50 years are enough time to turnover almost the entire capital stock of the society. About two-thirds of capital stock is usually in machines and equipment and about one-third in buildings and other structures. In Japan, the average renewal period for capital stock in business, the time it takes for machinery and equipment in an industry to be almost completely replaced, ranges from about 22 years in the textile industry down to ten years or less in such fast-moving industries as telecommunications and electrical machinery (see Table). In agriculture the estimated life span of cultivars in the United States is seven years for maize, eight for sorghum and cotton, and nine for wheat and soybeans[13], and most experts believe the life span of cultivars will grow shorter. Relative to greenhouse warming, turnover is also fast for nonmachinery capital stock, which includes buildings, pipelines, and so forth. According to recent surveys, in the Federal Republic of Germany in 1985 some 60 per cent of the stock is less than 20 years old and in the Soviet Union some 80 per cent is less than 20 years old (Fig. 2).

At first such figures may surprise us. But some reflection about the built environment relieves the surprise. Consider the office space in a modern city. Most of the space is in buildings completed in the last 20–30 years. These new buildings are filled with new equipment, for example, new telephone systems. Indeed, even older buildings are filled with modern equipment that did not exist 15 or 20 years ago. The same is true for supermarkets, restaurants and other stores. A large fraction of residential housing is similarly young and, in turn, filled largely with new domestic appliances of all kinds. If societies grow at a rate of 2–3 per cent per year, as the industrialized societies have for the past 150 years, then half of all capital stock will always be younger than 30 years old.

Probably the systems that take longest to build are infrastructures. Even these are constructed in less than a century[14]. Many infrastructure systems are (or should be) continuously reconstructed. For example, roads are repaved every 5–15 years, depending on use. The 7,000-kilometre canal system of the United States was almost entirely built in the 30 years between 1820 and 1850. More than 90 per cent of the 300,000-mile US railway network was laid in the 65 years between 1855 and 1920. The paving of virtually the entire six million-kilometre surface road system of the United States was accomplished between 1920 and 1985. The US interstate highway system was completed in about 30 years from the time that it was announced by President Eisenhower. It is interesting to consider whether climatic change could require any public works on this scale; coastal protection and interbasin water transfer would seem the most likely candidates.

Because capital stock continuously turns over on a time scale of a few decades, it will be possible to put in place much technology that is adjusted to a changing climate. This can be done without extraordinary measures given reasonably accurate information about the future. For the shortest lifetimes, even accurate information about the present climate will do.

CAPITAL STOCK RENEWAL

Industry	Renewal period
All industries	13.4
Manufacturing (all)	15.8
Electrical machinery	9.8
Transport machinery	13.2
Pulp and paper	13.7
General machinery	14.2
Chemicals	16.6
Food stuff	16.7
Steel and non-ferrous metals	21.1
Textiles	22.5
Non-manufacturing (all)	11.8
Service	8.1
Transport and telecommunications	10.7
Construction	11.9
Finance and insurance	12.8
Distribution	15.6
Real estate	15.8
Electricity, gas and water supply	15.8

Average renewal period for capital stock of business corporations in Japan by industrial sector, 1986-87 (ref. 22).

That perhaps 90 per cent of the global capital stock in the year 2040 will be built after 1990 does not diminish the significance of some long-lived structures. Action may be necessary to protect cities such as Venice, where preservation of historic buildings is the goal. In such cases, the process of replacement is not relevant.

The adaptations for long-term climatic change will probably mostly be the same as for other climate variation. The technologies, small and large, that buffer human activity over the long-term will be the same ones that mollify the difference between daytime and nighttime temperatures, protect against normal variability between days, shield from storms and hail, adjust to the seasons, and adapt to the wide range of climates where people already live.

No one has yet presented a radical innovation uniquely adaptive for the greenhouse effect. The main innovations directed at the greenhouse effect are so far organizational, in particular research groups in universities and government and assessment groups, such as the Intergovernmental Panel on Climate Change (IPCC). If the future greenhouse climate in any place will consist of climates that already exist somewhere on Earth, then many of the adaptations may look familiar.

Because the population of the world is imploding into cities, it seems logical that technologies that make cities habitable in unwelcoming climates will be among the technologies that are most important. Houston, Phoenix and Toronto may offer lessons. The trend in such places is toward ever larger enclosures of space and passageways connecting them, where workers and shoppers are not subject to the elements. Already during much of the year in such cities few people are seen outside on the streets. Technologies for 'smarter' buildings and for more efficient building materials should be adaptive. Cities in developing countries, which are often in difficult climates to begin with and face worsening problems of urban pollution, may well lack the resources to apply such technologies to raise or maintain the quality of life in the face of changing climate.

Until this century much of the human struggle with climate was to keep warm. Because the struggle succeeded, in 1850 the population in Europe, a land of well-chronicled and damaging winters, was three times as large as that of Africa and nine times as large as that of Latin America[15]. Now a main change in adaptation will be emphasis on technologies to stay cool. There is already pressure and success in this direction, population grows in tropical regions and people migrate south in temperate zones. Chemicals for refrigeration that do not exacerbate the greenhouse effect may thus earn a premium, and, of course, low greenhouse gas emission technologies to produce electricity and energy in general.

For water resources, larger-scale control of flows may be the trend. The Thames Barrage and the Netherlands Rhine Delta scheme may exemplify massive hydraulic systems that will be imitated in areas of major coastal populations. Fresh-water systems in some regions would also be made more robust by extending networks of supply over wider spaces through interbasin transfer and other strategies. In practical terms, many of the technologies needed may be well-established, for example, tunnelling, pumps and other technologies traditional to civil and mechanical engineering, updated with electronic sensors and other devices for management and control. Technologies for management of water demand will be equally or more attractive in many regions; these would include not only hardware technologies for minimizing leaks, but also software technologies from operations research, as well as economic and other incentives.

In agriculture, with a few notable exceptions, most emerging technologies are expected to reduce substantially the land and water required[16]. At least in the United States the trends in agricultural technology are in the direction that should be sought in view of climatic change. Almost all technologies that are attractive for agriculture are only more attractive in light of the possibility of climatic change. Specifically, appealing directions for agricultural innovation might include diversification of crop production by varying maturity, heat and drought tolerance, input needs, and end uses; innovation in planting and spacing; collecting and recycling irrigation run-off; soil moisture conser-

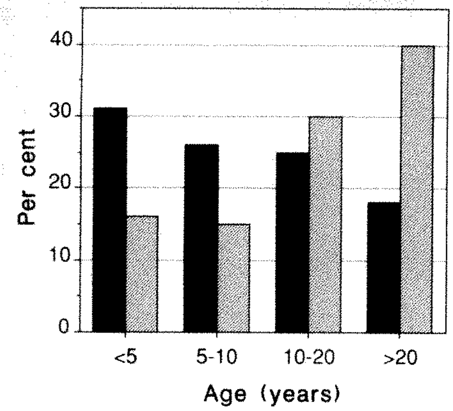


FIG. 2 Age distribution of nonmachinery capital stock in the FRG, data for 1985 (ref. 23) (hatched bars), and in the Soviet Union (solid bars), data as of 1986 (USSR State Committee on Statistics, Statistics on social indicators and capital vintage structure in industry, undated memo, Institute for Social and Economic Statistics, Moscow; courtesy of A. Grübler, Laxenburg, Austria).

vation; better moisture-use efficiency and improved use of plastics and other new materials; resistance to pests and insects; management practices; institutional measures; programmes and facilities to support extreme contingencies; and infrastructure. Many of these are applications of information technologies, as well as biotechnologies and more traditional agricultural and mechanical technologies. It may be possible to design and select plants adapted to higher concentrations of CO_2 and other changes in the atmosphere.

Though many innovations helpful in a rapidly changing climate are more likely to come from private enterprise than government, governments can help in two ways.

One way governments can aid adaptation is through timely information. One variety is assessments of the issues relating to climatic change. A second important and more operational variety of information is improved weather and climate forecasts. Eventually the climate of the far future will become tomorrow's weather. Information about it is likely to improve the possibility that it will be more resource than hazard.

There has been a gradual, measured improvement in weather forecasting during the past 20 years (ref. 17). In the greenhouse issue, all nations should find strong motivation to improve forecasts and the data and research underlying them. The quality of weather analyses and forecasts in many developing countries, especially in the tropics, is markedly lower than those in developed countries, particularly in the northern hemisphere. Advances in numerical modeling and extension of technology for monitoring in tropical regions can cause substantial improvements.

A third innovation in information relates to markets. The needs are for facilitation of information flows and improvements in rules for markets, in particular, markets for water. In many nations, water is allocated largely through administrative means based on water rights. Water transfers accommodate new patterns of climate, as well as changing farming and urban and industrial growth. They allow water to be used where it is most valuable[18]. Flexibility of water transfer is important, because the life of water projects is often 50–100 years or more[19].

Substantial subsidies for water for irrigation, in particular, lead to prices that encourage inefficiency. There is little incentive to conserve. Higher cost of constructing water projects and more demand and competition for water for such uses as preservation of wildlife, recreation, and cities make the issue serious. Several long-term contracts in the Central Valley of California provide water for only $3.50 per acre-foot, whereas new sources of supply would cost the federal government or the state $200–300 per acre-foot per year for construction alone[18]. Allowing and encouraging voluntary marketing of the resource among users could help adapt to climate changes and produce economic benefits. Voluntary water transfers could take several forms, including permanent sales, long-term leases, short-term leases, and leases contingent on drought.

Although markets may require innovation by government in providing information and rules, there are also traditional 'hardware' opportunities for innovation in public works. Government is the primary purchaser, financier and manager of systems of water supply and waste disposal, as well as coastal facilities. These take decades to site and construct and then can last generations. There may be opportunities for government to enhance innovation in infrastructure in light of the possibility of a changing environment.

Conclusion

Technologies are available for adaptation to climate on a spectrum of space, time and cost. Within minutes and for a few dollars one can buy an umbrella for local protection against a shower. Such technologies diffuse rapidly into the society, in a matter of months or years. Larger and more costly innovations, like electric refrigerators, may take 20 or 30 years to become pervasive. At the other end of the spectrum, large systems, like those for water and transportation take several decades or generations to extend themselves fully and may cost tens or hundreds of billions of dollars.

Technological performance has improved throughout human history, and in this century waves of innovation have come ever more rapidly[20]. In many systems, there have been steady improvements in efficiency of about two per cent per year, so that systems built today, for instance in energy, are about twice as efficient as those built 30 years ago[21]. Today generating a kilowatt of electricity in a steam plant takes only 15 per cent as much fossil fuel as at the turn of the century. A doubling of overall efficiency of several major systems should be possible just by replacing existing systems with best technologies and practices available today. This, of course, takes capital, and it is not clear that the expected rate of climatic change warrants an acceleration over the rate of change in physical capital stock that is already occurring, as long as the new stock is acquired with the best information about future climate in mind.

The general direction of change in technology and civilization is heartening for those anxious about climatic change. The trend is toward systems that are less vulnerable to climate. It would seem to be sensible to maintain this course and not to revert to reliance on such technologies as sailing ships and water mills that are more sensitive to climate. The highest need is probably to assure the inventive genius, economic power, and administrative competence that make the many technologies useful in adapting to climate available to the most people. ☐

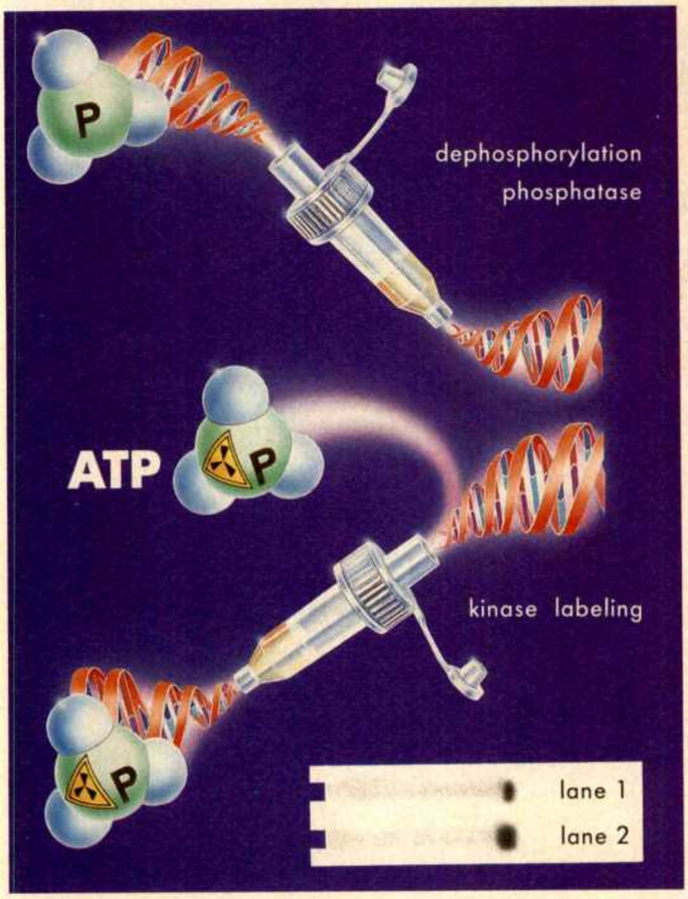
Jesse H. Ausubel is at The Rockefeller University, New York, New York 10021-6399, USA.

---

1. Nordhaus, W. D. *Setting National Priorities: Policy for the Nineties* (ed. Aaron, H.) 185-211 (Brookings, Washington, 1990).
2. Adams, R. McC. & Nissen, H. *The Uruk Countryside: The Natural Setting of Urban Societies* (University of Chicago Press, Chicago, 1972).
3. Newman, L. F. (ed.) *Hunger in History: Food Shortage, Poverty, and Deprivation* (Blackwell, Oxford, 1990).
4. Desmond, K. *Harwin Chronology of Inventions, Innovations, and Discoveries from Pre-History to the Present Day* (Constable, London, 1986).
5. Schelling, T. C. in *Changing Climate* (National Research Council) 449-482 (National Academy Press, Washington, 1983).
6. Escudero, J. C. *Climate Impact Assessment* (eds Kates, R. W., Ausubel, J. H. & Berberian M.) 251-272 (Wiley, Chichester, 1985).
7. Weihe, W. H. *Proceedings of the World Climate Conference* 313-368 (World Meteorological Organization, Geneva, 1979).
8. *New York Times*, 1 August 1990, p. C1.
9. Warrick, R. A. *Climatic Constraints and Human Activities* (eds Ausubel, J. & Biswas, A. K.) 93-123 (Pergamon, Oxford, 1980).
10. Reynolds, T. S. *Sci. Am.* **251**(1) 122-130 (1984).
11. Intergovernmental Panel on Climate Change *Scientific Assessment of Climate Change, Report to IPCC from Working Group 1* (World Meteorological Organization, Geneva, 1990).
12. US Bureau of the Census *Statistical Abstract of the United States: 1990* 110th edition (US Government Printing Office, Washington, 1990).
13. Duvick, D. N. *Econ. Botan.* **38**, 161-178 (1984).
14. Grübler, A. *The Rise and Fall of Infrastructures: Dynamics of Evolution and Technological Change in Transport* (Physica, Heidelberg, 1990).
15 McEvedy, C. & Jones, R. *Atlas of World Population History* (Penguin, New York, 1978).
16. Office of Technology Assessment, *Technology, Public Policy, and the Changing Structure of American Agriculture* (US Government Printing Office, Washington, 1986).
17. World Meteorological Organization (WMO) *The World Weather Watch: 25th Anniversary 1963-1988* (WMO No. 709, Geneva, 1988).
18. Wahl, R. W. *Markets for Federal Water: Subsidies, Property Rights, and the Bureau of Reclamation* (Resources for the Future, Washington, 1989).
19. Waggoner, P. E. (ed.) *Climatic Change and US Water Resources* (Wiley, New York, 1989).
20. Mensch, G. *Stalemate in Technology* (Ballinger, Cambridge, 1979).
21. Nakicenovic, N. & Grübler, A., *Technological Progress, Structural Change and Efficient Energy Use: Trends worldwide and in Austria, International Part* (International Institute for Applied Systems Analysis, Laxenburg, 1989).
22. Economic Planning Agency *Economic Survey of Japan, 1987-1988* (Tokyo, 1989).
23. Statistiches Bundesamt *Wirtschaft und Statistik* 4/89 (Metzler-Poeschl, Stuttgart, 1989).

Fast and Effective Labeling of Nucleic Acids

Labeling is easy with ready-to-use CR Columns containing immobilized PN kinase. Where necessary, prior dephosphorylation is achieved using immobilized alkaline phosphatase. The whole protocol takes less than 5 minutes. The tightly-bound enzymes are very stable, easy to use and cost effective since they can be re-used many times. Labeling with CR Columns is also more intensive, see gel – oligonucleotides ^{32}P-kinased in solution for one hour (lane 1) or with CR Column in 5 minutes (lane 2).



Distribution worldwide

Biometra, Göttingen	Tel.: 05 51-37 10 32
Dipl. Ing. F. Gatt, Innsbruck	Tel.: 05 12-58 30 17
Belgium, Netherlands, Luxembourg	
Sopar Biochem, Bruxelles	Tel.: 2 425 14 55
PolyLabo, Strasbourg	Tel.: 88 39 13 10
Biogenzia Lemania, Lausanne	Tel.: 021 25 40 11
Vias, Milano	Tel.: 2 415 17 21
AG Cientifica S.A., Madrid	Tel.: 803 11 50
Scotlab, Bellshill	Tel.: 0698 84 16 16
Norway, Danmark, Finland, Sweden	
KeboLab, Stockholm	Tel.: 8 621 34 00
Ambion, Austin	Tel.: 800 888 88 04
US Biochemicals, Cleveland	Tel.: 800 321 93 22
Funakoshi, Tokyo	Tel.: 03 293 23 67
Lord's Way Co., Taipei	Tel.: 02 563 88 30-1
Bio/Can Scientific, Mississauga	Tel.: 1800 38 78 125
Canadian Bio clinical Ltd.	Tel.: 416-293 49 55

Mo Bi Tec. We think harder – you work less.

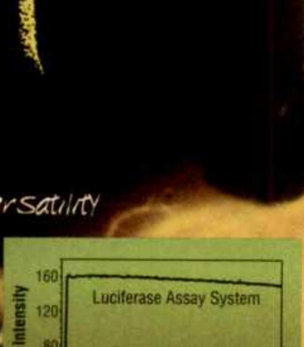
Mo Bi Tec

MOLECULAR BIOLOGISCHE TECHNOLOGIE

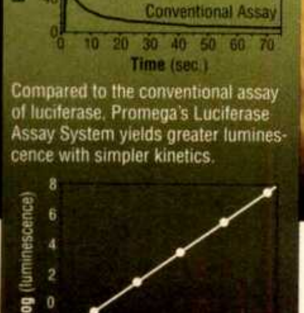
Mo Bi Tec GmbH, Wagenstieg 5, D-3400 Göttingen, Tel.: 05 51-37 10 62, Fax: 05 51-3 49 87

EUKARYOTIC EXPRESSION

Promega's GeneLight plasmids are mammalian expression vectors containing the luciferase gene with all combinations of promoter (P) and enhancer (E).



Compared to the conventional assay of luciferase, Promega's Luciferase Assay System yields greater luminescence with simpler kinetics.



Promega's Luciferase Assay System has a linear response over eight decades of reporter concentration.

To find out more about Promega's Luciferase product line, call us toll free or see our Luciferase Assay System Technical Bulletin 101, pGEM®-*luc* DNA Technical Bulletin 104, or GeneLight™ Reporter Plasmids Technical Bulletin 107.

Luciferase
Reporter Gene Technology

Luciferase Assay System
Firefly luciferase is widely recogniz as a superior reporter of genetic activity because its assay is rapid, extremely sensitive and non-isotopi Promega's Luciferase Assay System provides several advantages over th conventional assay method:
- **Increased luminescence – 10-fold or greater**
- **Simpler assay procedure – eliminat the need for automated injection devices and rapid mixing protocols**
- **No luminometer required – adaptab to scintillation counters or photographic films**

GeneLight™ Reporter Plasmids
Promega's four GeneLight reporter plasmids employ luciferase as a reporter gene for analysis of genetic promoter and enhancer elements. They offer:
- **Comparative analysis using analogo plasmids containing different genet elements**
- **Insertion of DNA sequences upstrea or downstream from the luciferase gene**
- **Analysis using nested deletions or site-specific mutagenesis**

pGEM®-*luc* DNA
pGEM-*luc* DNA provides a luciferase cassette with several unique cloning sites at both ends. Applications inclu analysis of:
- **Transcriptional regulation**
- **mRNA processing**
- **Protein structure/function analysis**
- **Labeling of cells and viruses**

Promega

Promega Corporation
2800 Woods Hollow Road
Madison, WI 53711-5399 USA
Toll Free 800-356-9526
Telephone 608-274-4330
Fax 608-273-6967
Telex 62057092

800-356-9526

Reader Service No. 690

Is Darwinism a thermodynamic necessity?

The concept of fitness has helped evolutionists avoid the accusation of tautology, but there is a case for asking that the notion should be defined in operational terms as well as by its consequences.

THE argument that the theory of natural selection is nothing but a tautology, due in its most elegant form to Sir Karl Popper, now hardly ever keeps evolutionists or population geneticists awake at night. That, no doubt, is how it should be. If artificial selection can be used to breed specialized forms of animals or plants, why should not natural selection, by the slings and arrows of the changing environment, yield species well-adapted to the world in which they find themselves?

That is a defensible position, but it does not remove the nagging doubt. Most simply, the tautology is the observation that if, in the "survival of the fittest", the fittest are identified with the survivors, the principle of natural selection amounts to the empty slogan "survival of the survivors". This proposition does not explain why there should be survivors anyway.

Evolutionists seek to avoid the difficulty by the intermediate concept of "fitness", a measure of the adaptation of an organism to the present environment that Darwin would have related to the numbers of living offspring it is likely to leave behind. There are many circumstances in which the notion works well and simply as "for example" in accounting for the survival of the sickle-cell gene in regions where malaria was once endemic; the relative immunity of heterozygotes to infection (compared with those carrying normal genes) outweighs the decrement of fitness of those carrying two copies of the gene, who are likely to suffer from congenital and often-fatal anaemia.

Latterly, fitness has been more generally defined as the likelihood that an organism will contribute genes to the future gene-pool, thus allowing for the evolution of behaviour patterns anthropomorphically called altruistic (and for the notion of the "selfish gene"), but the principle is the same. The trouble, however fitness is defined, is that it is a *post hoc* measure of the course of evolution; to make a theory of evolution, one has to enumerate all possible genetic changes, estimate the degree to which they are likely to be represented in the future gene-pool and only then to tell in what way a population is evolving. Is there not some way in which fitness in this context might be more directly defined by the degree to which the metabolic processes on which an organism depends for its survival are biochemically matched, or adapted, to the environment?

If there were such a generally applicable definition, it would be possible to prove that evolution is an unavoidable necessity or, more accurately, to identify the environmental parameters (and their rates of change with time) conducive to evolution. One source of confusion, but occasionally of conceptual difficulty as well, is that natural selection works on individual organisms, but that what evolves is a species or even an aggregation of species.

A further difficulty is that there is an unbridged gap between the mechanisms, necessarily biochemical at root, by which an organism can be counted well-adapted to its environment and the likely selection of its genes into the next generation's gene-pool. And as things are, there are not the physical criteria by which it can be judged whether the chance of selection is maximized.

This is the position from which J.-L. Torres, a Mexican from the University of Michoacan working at the International Centre for Theoretical Physics at Trieste, embarks on an attempt to apply the rules of nonequilibrium thermodynamics to the calculation of fitness in the mechanistic sense (*Il Nuovo Cimento* **13D**, 177; 1991). His objective is to find some way of measuring an individual's fitness by the degree to which its chief metabolic processes satisfy several thermodynamic criteria.

The working criteria are straightforward enough. A high degree of fitness would imply that metabolic processes are maximally efficient and, moreover, that they are maximally productive of whatever products the organism requires of them. On the other hand, the rate of entropy production should be a minimum, as should be the rate at which energy resources are consumed. In some circumstances, other criteria — for example, the rate of biomass production — might have to be reckoned with. By listing these criteria, Torres pretends no more than that they are a collection of plausible candidates in the construction of some composite indicator of what might be called instantaneous fitness.

But does not this make a horrendously complicated problem? The task of applying criteria such as these, not necessarily compatible with each other, to all the major metabolic cycles of an organism is evidently not child's play. But Torres is merely concerned to argue that it may be a little simpler than it seems.

Using such scant experimental data as there are for the production of ATP by the conversion of glucose into water (in red blood cells) and by respiration (in animal cells), he persuades himself, for example, that the expectations of conventional equilibrium thermodynamics do not necessarily apply. For then one would expect there to be a sharp conflict between the rate of ATP production and the efficiency of the process; the faster, the less efficient. In the classical Carnot cycle, the most efficient heat engine there can be, all processes are taken to be infinitely slow so that the power is infinitesimally different from zero. Why should ATP production be different?

In reality, Torres argues, in the nonequilibrium steady-states that prevail when ATP is produced in living organisms, physiological conditions correspond to a situation in which the measured efficiency of the reaction (measured as the fraction of free energy converted) is greater than a half and the rate of conversion is a maximum or nearly so.

In principle, at least, this points to a solution of this part of the problem. If the rate of ATP production and the efficiency of the process are to be regarded simultaneously as measures of fitness in the sense of matching the performance of an organism and the external demands of its environment, there will be a trade-off to be struck whose consequences are not nearly as depressing as page-one thermodynamics would lead one to expect.

That is a cheerful signpost, but not nearly half the real battle of defining, let alone calculating, some kind of dynamical instantaneous measure of fitness. Indeed, there is a what may be a little more than a procedural difficulty tucked away in an appendix in which Torres sets out to derive a general form for the velocity of a network of reactions catalysed by enzymes (which is a necessary step in calculating the flux of reactants and products in a steady-state). He starts from the elementary textbook statement of the law of mass action that the rate of an uncatalysed reaction is determined by the concentrations of the reactants each raised to an integral power equal to its stoichiometric coefficient, and then supposes that the effect of enzymes is to multiply each coefficient by a constant different from the number one.

Many biologists will be tempted to regard this as a pointless exercise, but they will be mistaken. What matters in, say, population genetics, may be the degree to which an organism, by the totality of its behaviour, contributes genes to the future gene-pool, but it is a laudable exercise to see whether that state of affairs is reflected in the way in which the metabolism of the organism is matched biochemically to the environment. And, for what it is worth, Torres admits that there are "nontrivial gaps in the argument", which is a seemly sign of modesty. **John Maddox**

SUPERNOVAE

Running rings around supernova 1987A

Ph. Podsiadlowski

THE bright nebula around the supernova SN1987A turns out to be a true ring of material and not an all-enveloping shell as had previously been assumed. Crotts and Heathcote come to this conclusion on page 683 of this issue[1], on the basis of high-resolution spectra of the nebula.

The good news four years ago was that a supernova had occurred sufficiently close for astronomers to get the first good view in four centuries of this final, violent demise of a star. The interesting news was that, in many respects the supernova did not fit astronomers' expectations for a classical type II supernova. One of the main puzzles of the event, which is still unresolved, is the question of why the immediate supernova progenitor was a blue supergiant rather than a red supergiant, as had generally been expected for this supernova type. Crotts and Heathcote's results on the ring around SN1987A fit this confounding trend and further constrain the models for the supernova's progenitor star.

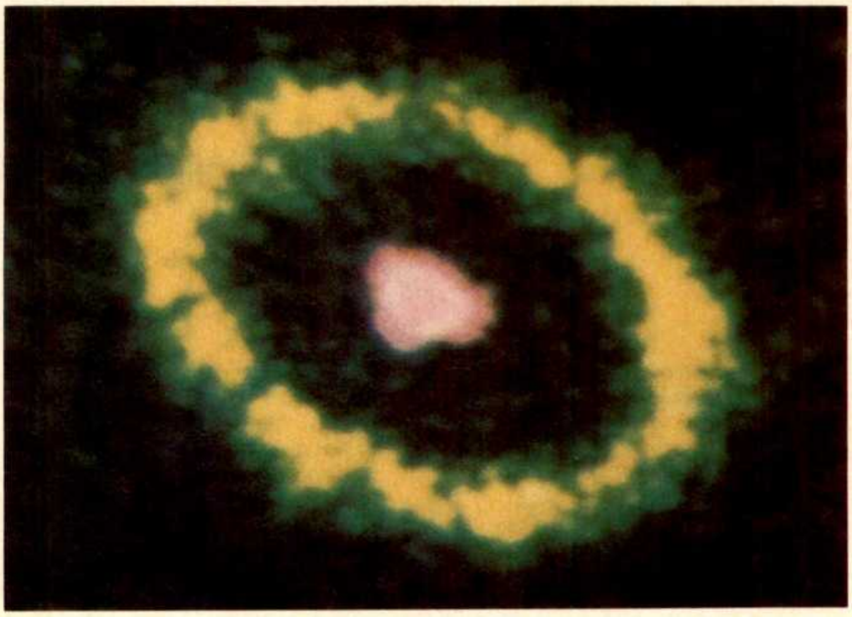


FIG. 1 The ring around supernova SN1987A as recorded by the Hubble Space Telescope on 23 August 1990. The ring was formed by the progenitor star before the explosion seen in 1987 and is illuminated by the supernova. The tightly knotted debris from the explosion appears as a red blob near the centre of the ring (Courtesy of NASA).

The presence of dense material around the supernova had been known since the early days of the explosion. It was first inferred from narrow emission lines in the ultraviolet spectrum[2]. The importance of this material was immediately recognized, as it represents the last structure generated by the progenitor star and thus provides a fingerprint of its late evolution. The large ratio of nitrogen to carbon and oxygen[2] and the small velocity spread of this material[3] indicate that it was processed by nuclear reactions deep inside the progenitor, was subsequently dredged up to the surface and ejected in a low-velocity wind when the progenitor was in its red-supergiant phase (which, in this picture, would have ended only 20,000 years before the explosion).

The surprise is that this material has the appearance of an elliptical ring[4,5] rather than that of a spherical shell as had been assumed previously (Fig. 1). In this issue, Crotts and Heathcote[1] present high-resolution spectra of the nebula, obtained over the past two years, in which the velocity structure across the nebula is spatially resolved. By comparing the line shapes of several prominent emission lines with simulated line shapes obtained from a simple dynamical model for the velocity structure of material in the nebula, they are able to demonstrate that the ring is indeed a physical ring (a circular ring of material inclined with respect to the line of sight); the nebula's structure is therefore very different from the spheroidal shells, often seen around the central stars of planetary nebulae, which superficially have the same ring-like appearance.

Although there can be little doubt left that we are seeing a physical ring of material formed before the supernova, its origin is, at the moment, rather mysterious. The ring-like geometry of the ejecta implies that the progenitor and/or its winds had an axisymmetric, but highly nonspherical structure.

Such an asymmetry could be produced if the progenitor was a rapidly rotating supergiant. But straightforward considerations of angular momentum show that even a star which rotated rapidly during its early evolution would become a slow rotator as it expanded into a supergiant phase[6]. Although a modest amount of rotation may be sufficient to explain the elliptical appearance of many planetary nebulae[7], it is very doubtful that it could produce the rather extreme deviation from spherical symmetry represented by a narrow ring.

On the other hand, rapid rotation could still provide an explanation for the ring. Th only ingredient that is missing is a source o angular momentum. A close binary companion could provide just such a source. It i neither implausible nor, *a priori*, unlikel that the progenitor of SN1987A was a member of a close pair of stars, as multiplicit among stars is the rule rather than the exception (the Sun being an obvious exception). I such a binary environment, there are severa mechanisms that could produce a ring. Th presence of a companion alone may be sufficient to gravitationally focus any mass los from the stars toward the pair's orbital plane[8] Alternatively, mas transfer between th stars in an interactin binary can provide th means by which orbita angular momentum o the binary's motion i transformed into spi angular momentum o the progenitor.

Several interacting binary models have previously been propose (see ref. 9) to explain th unusual features o SN1987A. Most fall int one of two classes: thos in which the progenitor i the result of the merge of two stars in a commo envelope, and those i which the progenito accreted a substantia amount of matter from close binary companion Although the detail vary substantially, all th models indicate tha after the mass-accretion phase or mergin process, the presupernova star would be rotating rapidly, probably have a thoroughl mixed envelope and be surrounded by a disl of material ejected in the interaction phase In the final blue-supergiant phase (which according to the analysis by Crotts an Heathcote[1], lasted only 20,000 years), th fast, energetic wind from the blue supergian would sweep up the material in this disk an form the dense ring we now see. It is wort emphasizing that models of this type can provide a theoretical framework in which thre of the principal unexpected features o SN1987A can be explained simultaneously the blue colour of the progenitor; the rin surrounding it; and the chemical anomalie in the progenitor's envelope[10].

Most binary models also make clear, testable predictions. In an accretion model, th companion of the progenitor has still to b present in the system and should make itsel conspicuous within the next few years. In merger model, where the progenitor woul have been a single blue supergiant at th time of the explosion, confirmation coul come from a detailed reconstruction of th

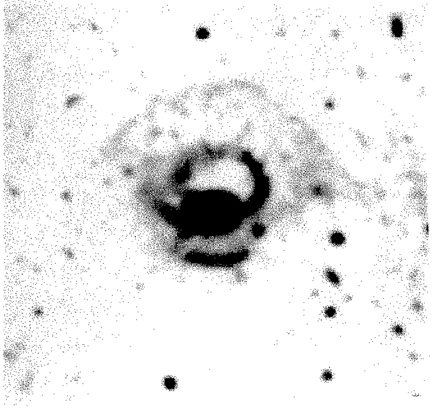


FIG. 2 The larger picture of the complex nebula around SN1987A, including the faint "Napoleon's hat". (From ref. 4.)

progenitor's history of mass loss.

In fact, the supernova ring is only part of a much larger nebula around the supernova[4] (Fig. 2). The appearance of the whole nebula is quite complex, but clearly structured (one of its major features has the appearance of Napoleon's hat). Because the whole nebula had to be generated by the star or the stars in the neighbourhood of the supernova, it provides a direct imprint of the progenitor's eventful past, and any model, single or binary, must be able to account for it.

As in any good mystery story, the clues are all out in the open; it is our job to decipher their meaning. This is where the next challenge in the modelling of this unusual supernova lies. While, for the moment, the ring may have added another puzzle to SN1987A, it also has provided some solid, new evidence which, in the long run, may lead us onto the right track and help to unravel this supernova's true pedigree. ☐

Ph. Podsiadlowski is in the Institute of Astronomy, University of Cambridge, Madingley Road, Cambridge CB3 0HA, UK.

---

1. Crotts, A. P. S. & Heathcote, S. R. *Nature* **350**, 683–685 (1991).
2. Fransson, C. *et al. Astrophys. J.* **336**, 429–441 (1989).
3. Wampler, E. J. & Richichi, A. *Astr. Astrophys.* **217**, 31–34 (1989).
4. Wampler, E. J. *et al. Astrophys. J.* **362**, L13–L16 (1990).
5. Jacobson, P. *et al. Astrophys. J.* **369**, L63–L66 (1991).
6. Chevalier, R. A. & Soker, N. *Astrophys. J.* **341**, 867–882 (1989).
7. Kahn, F. D. & West, K. A. *Mon. Not. R. astr. Soc.* **212**, 837–850 (1985).
8. Morris, M. in *From Miras to Planetary Nebulae: Which Path for Stellar Evolution?* (eds Mennessier, M. O. & Omont, A.) 520–535 (Editions Frontiers, Gif sur Yvette, 1990).
9. Hsu, J. J. L., Joss, P. C., Podsiadlowski, Ph. & Rappaport, S. in *Supernovae* (ed. Woosley, S.) 191–197 (Springer, New York, 1990).
10. Höflich, P. *Proc. astr. Soc. Australia* **7**, 434–442 (1990).

HOTSPOTS

Bermuda stretches a point

Randall M. Richardson

Hotspots, identified as the deep-seated cause of certain linear chains of volcanoes that get progressively older along their length, have played an important role in the study of plate tectonics since its earliest days[1,2]. With the Hawaiian–Emperor seamount chain as the classic and undisputed example, the number, origin and dynamics of hotspots have been hotly debated over the years. At one time, four hundred were postulated for Africa alone[3]. It seemed that workers correlated every large hill or linear volcanic feature with a hotspot. This trend has waned over time, as exemplified by new work by Peter Vogt[4] providing an alternative explanation for the volcanism and elevated topography of the eastern United States and along the Bermuda Rise in the western Atlantic. He postulates two northeast trending, linear, convective upwelling zones about 1,000 km on either side of the eastern North American continental margin, separated by a downwelling zone at the continental margin. The convection is presumably locked to the continental margin by deep thermal contrasts between continental and oceanic lithosphere.

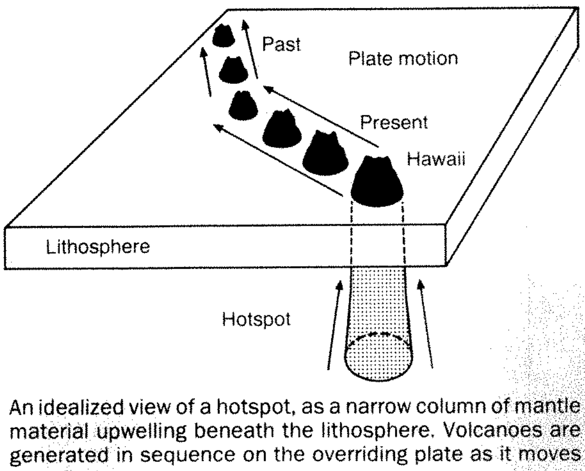
Hotspots, with sources beneath the lithosphere, have been an attractive explanation for long chains of oceanic seamounts, especially in the fast-moving Pacific plate. An idealized hotspot is pictured as a relatively narrow columnar zone, at most a few hundred kilometres in diameter, of hot mantle material ascending rapidly through the mantle from a source in the mantle beneath the plates, if not from the core–mantle boundary itself (see figure). As a plate travels over the hotspot, a chain of volcanoes is created in sequence. Hotspots are, of course, part of the mantle convection system, which includes the plates themselves: at some point, the distinction between hotspots and other forms of mantle convection blurs. This may well be the case in Vogt's new suggestion.

The simple hotspot model seems to work well for the Pacific. It does not work nearly as well for slow-moving oceanic areas, such as the North Atlantic. The origin of the Bermuda Rise, for example, is enigmatic. Oriented roughly parallel to the Atlantic margin, it is nearly 1,500 km long, rises 800–1,000 m above surrounding seafloor, and has other geophysical anomalies[5] consistent with a hotspot origin. The two most difficult observations to explain in terms of hotspots are the lack of subsidence since the cessation of active volcanism 30–25 million years ago and the northeast orientation of the rise, which is nearly at right angles to the predicted motion of the North American plate. Vogt explains these observations by hypothesizing a zone of convective upwelling paralleling the continental margin.

The hotspot model is even more difficult to apply on the continents, presumably because of the interaction between hotspot flow and continental lithosphere. The present-day topography of the Appalachian Mountains and the northeast extension of the topographic high into Newfoundland, called the Appalachian–Labrador Rise by Vogt, has been difficult to explain, with hotspots or otherwise. This average elevation of 1–2 km has persisted long after the initial construction of the mountains, even after erosion should have reduced the topography to a plain. To explain the topography, Vogt appeals to another, paired, upwelling zone on the continental side of the margin paralleling the upwelling beneath the Bermuda Rise. The upwelling zones are presumably separated by a downwelling convective zone.

Vogt's model is intriguing for a number of reasons. First, it would explain why the Bermuda Rise and the Appalachian Mountains run parallel to the continental margin. To date, a weak Bermuda hotspot, unable to burn a hole through the plate and forced to take advantage of previously existing structures, has been the best hotspot explanation for the orientation of the Bermuda Rise[6,7]. The northeasterly trend of elevated topography on the continent has been particularly difficult to explain in terms of hotspots as well. Second, convective upwelling could also account for the long persistence, with minimal subsidence, of the topography of the Bermuda Rise and the Appalachians.

The work of Gurnis[8] clearly indicates the important role that continental lithosphere may play in controlling convection in the mantle. In that modelling, continents tend to move toward, and stagnate above, cold downwelling zones. This inhibits subduction and may lead to continental breakup. These model results, however, do not support the existence of paired upwelling zones on either side of continental margins as proposed by Vogt. Furthermore, the return flow of material from subduction zones to mid-ocean ridges[9] in the mantle may smear out



An idealized view of a hotspot, as a narrow column of mantle material upwelling beneath the lithosphere. Volcanoes are generated in sequence on the overriding plate as it moves over the hotspot.

RÉSUMÉ

Notes from underground

THE search for cosmological dark matter has taken Japanese physicists into caves in a stone quarry 100 km north of Tokyo. There, S. Orito *et al.* (*Phys. Rev. Lett.* **66**, 1951-1954; 1991) installed plastic sheets, covering 2,000 m^2, and left them for 2 years, shielded from cosmic rays and solar flares. Their hope was that any passing superheavy elementary particles, if such things exist, would leave in the sheets tracks of atomic damage that could be revealed later by chemical etching. The method is sensitive to slow-moving particles weighing at least 10^{12} proton masses. Orito *et al.* found no sign of any superheavy particle and conclude that their abundance must be less than one for every 10^{29} photons in the Universe — a result which pushes the cosmological limit but does not yet rule out this putative form of dark matter.

Tail tales

COMPETITION in the female reproductive tract between sperm of different males is a matter not only of sperm numbers, as is recognized, but of their length (*Proc. R. Soc.* B**243**, 181-185; 1991). M. Gomendio and E. R. S. Roldan looked at the sperm of certain primates and rodents, dividing the animals into two groups — polyandrous species, in which females mate with several males in the course of an oestrus cycle, and in which sperm competition occurs, and the singular monandrous species. The sperm of polyandrous species, it turns out, are significantly larger, by virtue of the length of their tails. The authors also report that longer sperm can indeed swim faster than their shorter brethren, and may also be able to penetrate the egg more easily — that is, size does matter.

Gender gap

R. C. GUR and colleagues, writing in *Proceedings of the National Academy of Sciences* (**88**, 2845-2849; 1991), show that men are more prone to certain age-related changes of the brain than are women. Gur *et al.* studied the crania of 69 healthy adults between the ages of 18 and 80 years using magnetic resonance imaging, and found support for the idea that ageing is, as previous work has shown, associated with a decrease in brain volume and increase in that of the cerebrospinal fluid. But they also observed that the rate of increase in volume of the cerebrospinal fluid is greater with age in men than it is in women. Moreover, men display an asymmetric effect, not seen in women, atrophy of the left hemisphere being more pronounced than that of the right. Choosing a judiciously conditional turn of phrase, the authors say that their study "may help us to understand neural substrates of behavioral changes associated with aging".

---

any features that might form at continental margins. A final question involves the long-term persistence of the upwelling zones. The distance of the paired upwelling from the mid-ocean ridge must change as new plate is created at the mid-ocean ridge. Thus, to persist, the upwelling must be very strongly tied to the thermal contrasts between continental and oceanic lithosphere.

The final, perhaps semantic, question is: when does a hotspot cease to be a hotspot?; or, what is the difference between hotspot convective upwelling and other forms of convective upwelling? If hotspots are limited to narrow upwelling zones moving slowly, at best, with respect to one another, then the distinction is rather straightforward. Vogt's upwelling has a long horizontal axis parallel to the continental margin and is tied to the continent rather than to other hotspots. How does this differ from a weak hotspot that gets smeared out horizontally? Is the distinction important?

Vogt's interesting argument may help explain features parallel to the continental margin, and further define hotspots. Vogt is correct in saying that one of the most important problems is the post-mountain building uplift history of the Appalachians, and that it will be hard to test his model. ☐

Randall M. Richardson is in the Department of Geosciences, University of Arizona, Tucson, Arizona 85721, USA.

1. Wilson, J. T. *Nature* **197**, 536-538 (1963).
2. Morgan, W. J. *Nature* **230**, 42-43 (1971).
3. Burke, K. & Wilson, J. T. *Nature* **239**, 387-390 (1972).
4. Vogt, P. R. *Geology* **19**, 41-44 (1991).
5. Detrick, R. S., Von Herzen, P. R., Sandwell, D. & Dougherty, M. *J. geophys. Res.* **91**, 3701-3723 (1986).
6. Crough, S. T. *A. Rev. Earth planet. Sci.* **11**, 165-193 (1983).
7. Liu, M. & Chase, C. G., *J. geophys. Res.* **94**, 5571-5584 (1989).
8. Gurnis, M. *Nature*. **332**, 695-699 (1988).
9. Chase, C. G. *Geophys. Jl R. astr. Soc.* **56**, 1-18 (1979).

BIOPOLYMERS

Light on molecular recognition

Robert Kaiser, Tim Hunkapiller and Leroy Hood

WRITING in *Science*[1], Fodor *et al.* describe an exciting strategy for the light-directed, spatially addressable parallel synthesis of a large number of chemically related yet distinct species on a glass surface — thus producing molecular diversity through combinatorial chemical synthesis. The technique provides a fresh way of tackling one of the fundamental problems of modern biology, namely molecular recognition, or how nucleic acids and proteins interact in a highly specific manner.

Interactions between nucleic acids generally arise from one-dimensional complementarity through the ability of nucleotides to form classic Watson–Crick base pairs. Molecular recognition at the DNA level, therefore, is often assayed by determining whether a DNA probe and target sequence hybridize. In contrast, proteins interact with other molecules by three-dimensional complementarity, the matching of shapes that give rise to optimal interatomic interactions.

Because the rules for protein folding are unknown, studies of molecular recognition at the protein–protein or protein–nucleic acid levels often centre around the testing of many diverse forms of one component for specific binding to the second component. In general the process can be broken into two parts: first, the generation of numerous forms in which a limited set of elements are combined in all possible ways to generate a potentially very large and diverse set of candidates for testing; second, the selective enrichment of one or a few high-affinity forms from this test set. In the past, investigators have tended to employ biological strategies for both combinatorial diversification and selection[2,3]. More recently chemical synthesis has been used to generate combinatorial diversification followed by biological selection[4]. Fodor's approach provides a massively multiplexed assay system that is no longer dependent on the limitations of competitive selection.

The power of combinatorial strategies for generating molecular diversity is best illustrated by the vertebrate immune system[5]. The cells of the immune system must be able specifically to detect and bind a pool of foreign molecular species (antigens) of potentially unlimited structural diversity. The genome of a single organism cannot hope to encode a unique receptor for every possible antigen, so the limited diversity of the germline genetic information must be amplified. This is accomplished by randomly selecting and combining elements from limited pools of gene segments (V, D and J) to construct a pair of unique rearranged genes for each immune cell that encodes the immune receptors (antibodies and T-cell receptors). Thus, the potential receptor diversity of an individual organism is proportional to the product of the sizes of the germline repertoires of the various gene-segment groups[5]. It is estimated that the human immune system has the potential to generate some 10^{11} unique antibodies by this and other combinatorial and/or mutational mechanisms[6]. Selection is then accomplished through clonal amplification of only those cells displaying a single type of receptor that complements an inducing antigen.

One particularly productive area of research that rests upon the antibody model has been the design and generation of antibody structures which mimic enzymes as selective catalysts[2,3]. Better understanding of enzymic catalysis has allowed the generation of specific antibodies displaying a wide variety of catalytic capabilities, including hydrolysis or formation of ester and amide bonds, acyl group transfer, β-elimination, carbon–carbon bond formation, porphyrin metalation, peroxidation and redox reactions. For example, antibody catalysts which mimic hydrolytic enzymes can be obtained using a transition-state analogue as the antigen to select for antibody-binding sites containing the appropriate amino-acid side-chain functionalities for transition-state stabilization and catalytic function. A powerful new *in vitro* combinatorial technique for generating a large repertoire of monoclonal antibodies for rapid screening for catalytic activity employs a combinatorial library of antibody recognition fragments in phage lambda[7].

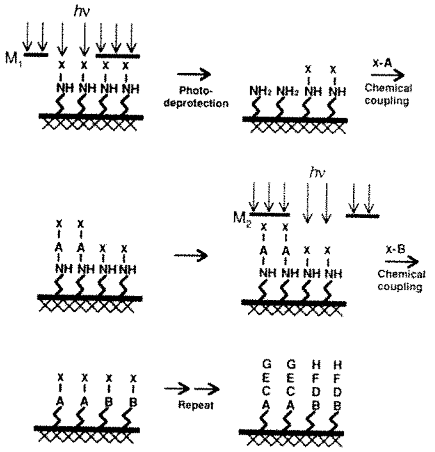
Combinatorial diversity followed by selection can also be applied to the study of almost any protein–ligand interactions in a manner directly analogous to *in vivo* antibody selection. Tuerk and Gold[4] have developed an approach based on the selective amplification of preferred protein-binding sequences from a large pool of DNA sequences. A 110-base oligonucleotide was chemically synthesized, incorporating completely random nucleotides in an eight-base region known to interact with T4 DNA polymerase when transcribed into RNA. The resulting pool of over 65,000 discrete DNA sequence variants was then enriched for those containing high-affinity binding sequences by first transcribing the DNA templates into RNAs, treating the resulting RNA pool with a small amount of the protein, capturing protein-bound RNA species on nitrocellulose filters, reverse transcribing the bound RNA into complementary cDNA, and subjecting the cDNA pool to amplification by the polymerase chain reaction (PCR). The PCR products were then transcribed into RNA and the process was repeated for four rounds.

Two sequences had been selectively amplified, one with the wild-type octamer sequence, and one differing from the wild type at four unpredictable positions. Thus, diversity generated by combinatorial chemical synthesis followed by selection of high-affinity conjugates can delineate optimal binding structures. Not only does this provide a means by which to develop a deeper understanding of specific protein–ligand interactions, it should also provide insight into the fundamental problem of predicting the partial three-dimensional conformation of proteins from primary sequence information.

A difficulty with relying upon competitive selection to assay for high-affinity structures is that only the very few exhibiting optimal interactions are isolated. For some purposes, the characterization of a set of interactants exhibiting a descending degree of affinity or quality of interaction, as provided by the spatially addressable synthetic system[1], could provide a more complete picture of the interactions.

In their report[1], Fodor *et al.* describe an intriguing new approach to the chemical generation of protein or nucleic acid sequences. The keys to the process are the elegant implementation of fairly conventional solid-phase chemistry, as well as the clever adaptation of photolithographic technology.

A glass slide is first derivatized with an appropriate silane to coat the surface with primary aliphatic amino groups, which are subsequently rendered unreactive with a photo-removable protecting group (see the figure). Light is then used to address discrete regions of the slide by means of photo masks in a process analogous to the photolithographic techniques employed in semiconductor circuit formation. Upon illumination, only unmasked areas have the protecting group removed and are thus activated for further reaction. The patterns described by the masks used for each illumination step define the location of each synthetic product, while the molecular nature of each product is determined by which reactants are added between successive maskings. The authors demonstrate that by using simple binary masking, one of many combinatorial synthesis strategies, $2n$ different polypeptide chains can be synthesized in n chemical steps using n masks. They also provide preliminary evidence for the chemical synthesis of oligonucleotides using this strategy.



The principles of light-directed, spatially addressable parallel chemical synthesis as described by Fodor and colleagues (reproduced from ref. 1). Amino groups attached to a glass slide are protected by a photolabile agent, x. When some groups are exposed to light through a mask (M_1) they become open to coupling to chemical building blocks (A), again each protected by a photolabile agent. The next mask (M_2) allows illumination to deprotect different amino groups, and for a different chemical building block (B) to be coupled to them. The cycle of masking, illumination and removal of the photolabile protecting agent, and then addition of further building blocks (C,D,E ...) to the exposed amino groups, continues until the products required have been synthesized.

Very high densities of biopolymer species can be achieved by using masks containing sufficiently small (tens of microns by tens of microns) elements. Thus, a set of thousands of different biopolymers can be synthesized on a single glass slide. Furthermore, Fodor *et al.* show that it is possible to interrogate the various spatial elements of the array using epifluorescence microscopy in conjunction with appropriate fluorescently labelled probes. So, not only can an enormous number of variants be assayed at once, but the specific result of each individual assay can be ascertained.

An addressable system such as this has a variety of potential applications. One is in the investigation of ligand–receptor interactions, both in the exploration of basic biological processes and in the design and testing of molecules for therapeutic or diagnostic applications. Another might be the sequence analysis of DNA, using the hybridization strategy proposed by Khrapko *et al.*[8] in which a series of fragments of template DNA are hybridized to a sorted set of all possible octamer sequences (a set with 65,256 members). It is proposed that by adjusting conditions to disallow even single nucleotide mismatches, the template sequence can be mathematically determined from the sequences of octamers forming stable hybrids.

The covalently bound biopolymer arrays thus made possible could have several advantages. The high densities obtainable provide the prospect of multiplexing tens of thousands of investigations, and it should also be possible to re-use the same array to perform several sequential assays interspersed with appropriate washes. Also, a variety of highly sensitive methods of detection (radioactivity, fluorescence, chemiluminescence, enzyme-linked reactions) could be adapted to this format fairly easily.

The method as described does suffer from some limitations, however. Repetitive yields of the peptide assembly reactions, for example, are only 85–95 per cent, thus restricting the maximum practical size of polypeptides which could be usefully generated. Additionally, the method does not appear to be viable for the preparative production of multiple biopolymers.

It is unclear from the data and descriptions provided by Fodor and colleagues just how difficult or how rapid it would be to carry out either a very large or several very small investigations into ligand–receptor interactions. However, the method they describe indeed holds great promise, which will be fully realized as powerful alternative technologies for the spatial addressing of discrete areas by light are developed. ☐

Robert Kaiser, Tim Hunkapiller and Leroy Hood are in the Center for Molecular Biotechnology, an NSF Science and Technology Center, California Institute of Technology, Pasadena, California 91125, USA.

---

1. Fodor, S. P. A. *et al. Science* **251**, 767–773 (1991).
2. Shokat, K. M. & Schultz, P. G. *A. Rev. Immun.* **8**, 335–363 (1990).
3. Lerner, R. A. *et al. Science* (in the press).
4. Tuerk, C. & Gold, L. *Science* **249**, 505–510 (1990).
5. Hunkapiller, T. & Hood, L. *Adv. Immun.* **44**, 1–63 (1989).
6. Davis, M. M. & Bjorkmann, P. J. *Nature* **334**, 395–402 (1988).
7. Huse, W. D. *et al. Science* **246**, 1275–1281 (1989).
8. Khrapko, K. R. *et al. FEBS Lett.* **256**, 118–122 (1989).

HIV

Tampering with transcription

Gary J. Nabel

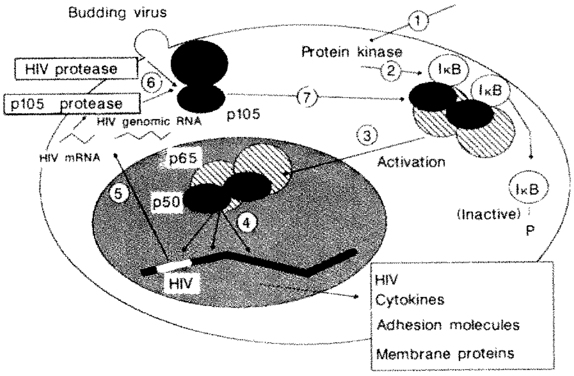
CONCEALED within a cell, the human immunodeficiency virus (HIV) quietly subverts a host that would otherwise seek to destroy it. Productive HIV infection reflects a delicate balance between the needs of the host cell and the demands of the virus. It is now clear that this balance has not been left simply to chance — two studies, one that appeared in last week's *Nature*[1], the other reported on page 709 of this issue[2], show that sophisticated strategies have evolved to alter cellular transcription and facilitate viral replication. Many cellular proteins contribute to the replication of HIV, including NF-κB, an inducible transcription factor that binds to regulatory sites in the HIV enhancer sequence and increases viral transcription after T-cell activation[3]. It now seems that NF-κB is also specifically induced during the course of infection[1], and that it may be regulated by a viral gene product, the HIV-1 protease[2].

Like all viruses, HIV is an intracellular parasite. Beginning with its entry into its host cell by way of the CD4 glycoprotein, the virus interacts with many cellular proteins to complete its life cycle. The importance of these host-cell factors became apparent during early attempts to isolate the virus. Propagation was much more successful when the host T cell was activated by mitogens[4,5], which causes an increase in NF-κB binding to DNA; and the two κB elements in the HIV-1 regulatory region, although not indispensable for replication[6], are responsible for the increase in stimulation through the enhancer sequence seen in activated T cells.

In cells infected by HIV, including T cells and some monocytes, NF-κB resides within the cytoplasm in an inactive form, complexed to an inhibitor, IκB. Cellular activation by mitogens or cytokines releases the active DNA-binding complex, which is composed of distinct DNA-binding (p50) and transactivation (p65) subunits (see figure). This process is regulated at several levels — phosphorylation by protein kinases, for example, causes IκB to disengage from the p50/p65 complex[7]. Another regulatory step probably involves the DNA-binding subunit of NF-κB. This protein is synthesized as a precursor of relative molecular mass 105,000 (M_r 105K) and requires processing to generate the active binding protein of M_r 50K.

Bachelerie and co-workers have now shown that infection by HIV stimulates binding of NF-κB to DNA[2]. In a promonocytic line, infection leads to a marked increase in NF-κB binding activity and function, raising the possibility that NF-κB has a part in perpetuating the infection and that viral genes activate NF-κB. Several mechanisms could account for the increase in binding activity; the induction of cellular protein kinases, transcriptional activation of p105 and/or p65, inhibition of IκB synthesis or activation of a protease might all serve this function. Rivière *et al.*[1] provide evidence for an intriguing possibility: the HIV protease, thought normally to regulate maturation of the virus by cleavage of the HIV polyprotein within budding forms, might also contribute to NF-κB activation. They have detected a smaller form of the processed p105 DNA-binding subunit. This smaller protein, of M_r 45K, is also generated by cotransfection with the HIV protease, and a potential aspartyl protease cleavage site has been identified which could give rise to this protein. Incubation of p105 NF-κB with the HIV protease *in vitro* also generates the protein. Normally, a yet undefined cellular protease is likely to serve this function.

These findings are an elegant example of the precision of viral/host cell interactions, and raise additional questions about the molecular activation of HIV during infection and the role of NF-κB. The HIV protease has been thought to be confined to the budding virus particle. This assumption must now be reassessed. Another possibility is that the virus particle can deliver sufficient protease to the cell to stimulate proteolysis of p105. At the same time, the possibility remains that HIV activates other cellular proteins, including protein kinases, cellular proteases or IκB, which regulate NF-κB.



Mechanism of NF-κB activation and its relationship to HIV infection. (1) Cells are activated by cytokines or mitogens. (2) Cellular protein kinases are induced, causing phosphorylation of IκB and other potential regulatory proteins. The phosphorylated form of IκB does not complex to p50/p65. (3) Dissociation of p50/p65 from IκB allows its translocation to the nucleus. (4) Nuclear NF-κB stimulates increased transcription of HIV and other cellular genes, including those responsible for cytokines, adhesion molecules and cell-surface glycoproteins. RNA (5) Viral RNA is synthesized, including messenger RNAs encoding structural proteins, such as the HIV protease, and genomic HIV RNA. (6) Activation of the HIV protease stimulates cleavage of the p105 precursor of the DNA-binding subunit of NF-κB. Infection or cellular activation could also induce cellular proteases, including the endogenous p105 protease. (7) The p50 DNA-binding subunit is incorporated into the p50/p65 complex.

Taken together, the observations raise the possibility that NF-κB maintains viral replication once it has been initiated. How might this occur? The most likely explanation is that NF-κB participates in the activation of a cellular gene required for later steps in the infectious cycle, and there is no shortage of cellular genes which could serve this function. The cytokine TNF-α, for example, stimulates NF-κB binding activity and HIV replication, and it has κB regulatory elements that regulate its expression. Although Bachelerie *et al.* found no extracellular TNF-α, the cytokine might act in an autocrine or intracrine fashion[8]. Many cellular genes could also code for proteins having this role, including other cytokines, growth factor receptors, adhesion molecules and membrane proteins which can be regulated by NF-κB. Possibly, it is not a single gene, but a set of coordinately activated host cell genes, which facilitates infection.

The alternative of NF-κB binding activity simply being a consequence of infection and having no active role in infection seems less likely. In addition to its association with HIV infection, the κB regulatory element has been found in many primate viruses and NF-κB is induced during their course of infection[9]; HTLV-I and Epstein–Barr virus, for example, contain specific viral transactivators which stimulate κB-dependent transcription by different mechanisms (refs 10 and 11; M.-L. Hammarskjold, personal communication).

Apparently, there is a common theme in infection by these different viruses which is not yet understood. As viral genes help to define critical steps in the regulation of cellular gene expression, these new insights may allow us to learn what the virus has already discovered. ☐

Gary J. Nabel is in the Howard Hughes Medical Institute, University of Michigan Medical Center, Ann Arbor, Michigan 48109, USA.

---

1. Rivière, Y., Blank, V., Kourilsky, P. & Israel, A. *Nature* **350**, 625–626 (1991).
2. Bachelerie, F., Alcami, J., Arenzana-Seisdedos, F. & Virelizier, J.-L. *Nature* **350**, 709–712 (1991).
3. Nabel, G. & Baltimore, D. *Nature* **326**, 711–713 (1987).
4. Zagury, D. *et al. Science* **231**, 850–853 (1986).
5. Harada, S. *et al. Virology* **154**, 249–258 (1986).
6. Leonard, J. *et al. J. Virol.* **63**, 4919–4924 (1989).
7. Ghosh, S. & Baltimore, D. *Nature* **344**, 678–682 (1990).
8. Poli, G. *et al. Proc. natn. Acad. Sci. U.S.A.* **87**, 782–785 (1990).
9. Gimble, J. M. *et al. J. Virol.* **62**, 4104–4112 (1988).
10. Leung, K. & Nabel, G. J. *Nature* **333**, 776–778 (1988).
11. Ballard, D. W. *et al. Science* **241**, 1652–1655 (1988).

SOLID-STATE PHYSICS

Glasses come to order

Raymond Jeanloz and Quentin Williams

As kinetically frozen forms of liquid, glasses are characterized by a complete lack of long-range crystalline order and are the most structurally disordered types of solid known. To be sure, short-range order is typically present among nearest atomic neighbours in glasses. But the inability to resolve medium- and long-range interatomic correlations has prevented glasses and glass properties from being clarified at the microscopic level at which the crystalline state is understood[1]. It is therefore a remarkable achievement that Gaskell *et al.*, who report on page 675 of this issue[2], have obtained the first experimental evidence for intermediate-range structural order in an oxide glass.

Glass is of widespread importance for several reasons, not the least of which are its technological applications. It is the lack of an underlying crystal structure that ultimately makes glass malleable — both mechanically, in that objects of nearly unlimited shapes can be formed, and also in the sense that electronic and other properties of glasses can be varied to best advantage for a given application. At a more basic level, there is much interest in the mechanisms by which condensed matter can become disordered. Glasses may offer significant insights into the structures and properties of melts, for example.

The archetypal glass, fused silica, is classically modelled as a continuous but disordered ('random') network of corner-linked SiO_4 tetrahedra. That is, order is considered to vanish only at intermediate to long ranges, beyond the first few coordination shells, because the tetrahedra are exceptionally strong and well defined molecular building blocks. Alkali and alkaline earth ions, which are often present in commercial glasses, are treated as 'network modifiers'. They are thought to disrupt the tetrahedral network, enhancing the disorder of the glass by counteracting the effects of the network-forming ions such as silicon.

The interplay between structure and composition has led to a rich literature in which the degree of polymerization, or the interconnectedness, of tetrahedral units have been documented for a wide variety of glasses[3]. Much of the evidence, necessarily indirect, comes from spectroscopic probes and has led to relatively detailed but arcane descriptions of glass structure. For example, much effort has gone into characterizing the abundances of Q^i species for $i \sim 0–4$, with Q^i being a cluster consisting of a network-forming tetrahedron corner-linked to i neighbouring tetrahedra[4]. How the Q^i speciation is related to glass or liquid-state properties is as yet unclear, but the consensus has been that at least this is a parameter that can be estimated from the observed spectra.

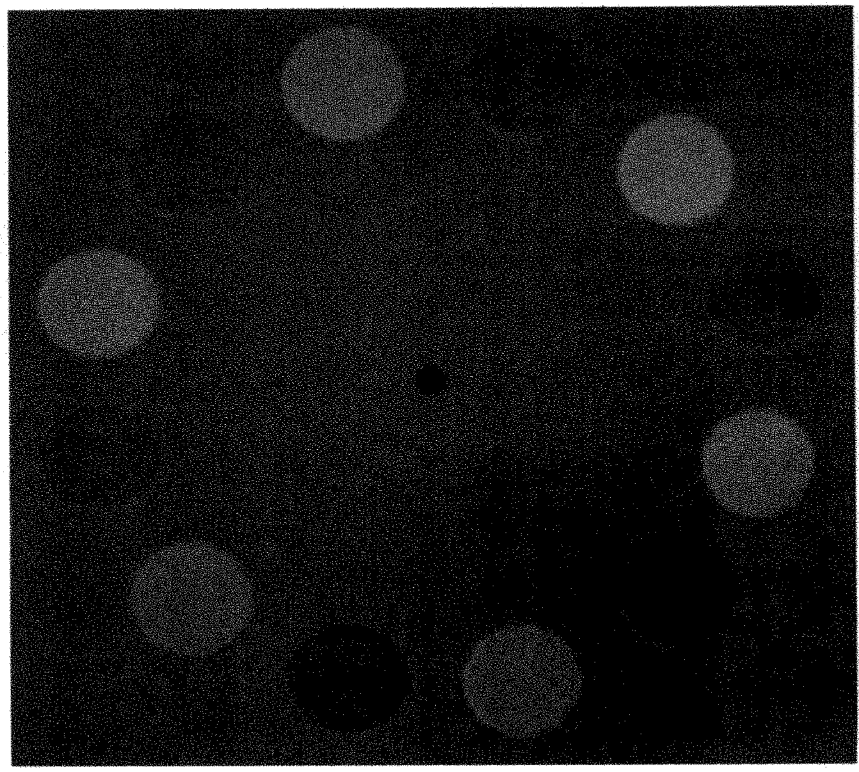
Now Gaskell *et al.* present new data which overturn the classical view of glass structure. Specificially, they demonstrate that far from enhancing the disorder of the structure of $CaSiO_3$ glass, the network modifier, calcium, itself exhibits medium-range ordering. To show this, Gaskell and co-workers have carried out an elegant neutron-scattering experiment. By examining samples with varying isotopic contents of Ca, they obtain diffraction profiles that differ because the neutron scattering cross-section depends on isotopic composition. Appropriately subtracting the resulting diffraction profiles from each other, they are able to experimentally determine not only the pair correlation function around Ca, which is dominated by the distribution of Ca—O bond lengths, but also the Ca—Ca correlation function.

The striking feature is that two strong peaks are evident in the latter, and these can be neatly explained as being due to first- and second-neighbour Ca—Ca distances within a sheet of CaO_6 octahedra. As in the crystal, the octahedra are edge-linked. Specifically, Gaskell *et al.* show that the first Ca—Ca distance and coordination number for the glass overlap with the crystalline values; in contrast, a random distribution of network-modifying Ca should yield a distance about 15 per cent longer.

Through these measurements, Gaskell and colleagues identify a serious shortcoming of the classical model of oxide glass structure. Instead, they propose a model based on a nearly close-packed arrangement of oxygen ions, with the cations filling appropriate octahedral and tetrahedral interstices. This model, which gives them the sheets of edge-linked octahedra required to explain the experimental data, also has a venerable history; but for describing crystalline rather than glass structures. In brief, the new data strengthen our appreciation for the close similarity between crystalline and glass structures, at least at short to intermediate ranges.

The idea that glass and crystal structures can be closely related is supported by recent investigations of an entirely different process for making glass, pressure-induced amorphization. In this method, a crystal is compressed to pressures beyond its range of stability, the result being that it is directly converted to a glass, without melting or even heating[5]. Remarkably, many such pressure-formed glasses become crystalline again on decompression[6–8]; and in one case, we found that a crystal that was amorphized under pressure reverted, upon decompression, to a

Spot the difference



If the eyes are focused on the dot in the middle of the central black spot, after 10—15 seconds some of the peripheral coloured disks disappear and the space they occupy becomes 'filled in' by the background. This effect, the result of "an artificial perceptual scotoma [blind spot]", is described by V. S. Ramachandran and R. L. Gregory on page 699 of this issue. It is, they say, the effect supposedly used by King Charles II to 'decapitate' his courtiers, using his natural blind spot.

single crystal with the original orientation[9]. Hence, the crystalline and amorphous structures are thought to be very closely related, with the amorphization perhaps being due to a crystal-structural instability involving small displacements of the ions.

The discovery that glasses have intermediate-range order opens up the prospect of tailor-making glass structures of a given compound. Indeed, there already exists evidence that distinguishable 'polymorphs' of glass — varying mainly in medium-range order — can be reproducibly made[10-14]. By analogy with the polymorphism of crystalline solids, the properties of such amorphous phases should be significantly altered as the structure is changed. The potential exists, therefore, for optimizing mechanical, optical and other properties of glass by varying its newly discovered structure. ☐

Raymond Jeanloz is in the Department of Geology and Geophysics, University of California, Berkeley, California 94720, USA and Quentin Williams is in the Institute of Tectonics, University of California, Santa Cruz, California 95064, USA.

1. Waseda, Y. *The Structure of Non-Crystalline Materials* (McGraw-Hill, New York, 1980).
2. Gaskell, P. H., Eckersley, M. C., Barnes, A. C. & Chieux, P. *Nature* **350**, 675–677 (1991).
3. Mysen, B. O. *Structure and Properties of Silicate Melts* (Elsevier, Amsterdam, 1988).
4. Stebbins, J. F. *Nature* **330**, 465–467 (1987).
5. Hemley, R. J., Jephcoat, A. P., Mao, H. K., Ming, L. C. & Manghnani, M. H. *Nature* **334**, 52–54 (1988).
6. Fujii, Y., Kowaka, M. & Onodera, A. *J. Phys.* C**18**, 789–797 (1985).
7. Sankaran, H., Sikka, S. K., Sharma, S. M. & Chindambaran, R. *Phys. Rev.* B**38**, 170–173 (1988).
8. Meade, C. & Jeanloz, R. *Geophys. Res. Lett.* **17**, 1157–1160 (1990).
9. Kruger, M. B. & Jeanloz, R. *Science* **249**, 647–649 (1990).
10. Mishima, O., Calvert, L. D. & Whalley, E. *Nature* **314**, 76–78 (1985).
11. Grimsditch, M. *Phys. Rev. Lett.* **52**, 2379–2381 (1984).
12. Tse, J. S. & Klein, M. L. *Phys. Rev. Lett.* **58**, 1672–1675 (1987).
13. Hemley, R. J., Chen, L. C. & Mao, H. K. *Nature* **338**, 638–640 (1989).
14. Williams, Q., Knittle, E., Reichlin, R., Martin, S. & Jeanloz, R. *J. geophys. Res.* **95**, 21549–21563 (1990).

NEUROBIOLOGY

trking neurotrophic receptors

Cliff Ragsdale and Jim Woodgett

A MOLECULAR collision has occurred between the fields of neurotrophic factor research and protooncogene biology. On page 678 of this issue[1] and in the 5 April issue of *Cell*[2], the *trk* protooncogene has been identified as the elusive 140K receptor for nerve growth factor that is required for high-affinity binding.

Nerve growth factor (NGF) was the first discovered and remains the best characterized of the target-derived neurotrophic factors[3]. It is a survival factor for sympathetic and selected sensory neurons of the peripheral nervous system and for injured cholinergic neurons of the basal forebrain; in addition, it can regulate the outgrowth of neuronal processes and promote the expression of neural differentiation markers. The factor may have clinical relevance in the treatment of neurodegenerative disorders, so elucidation of its mechanism of action has long been a priority.

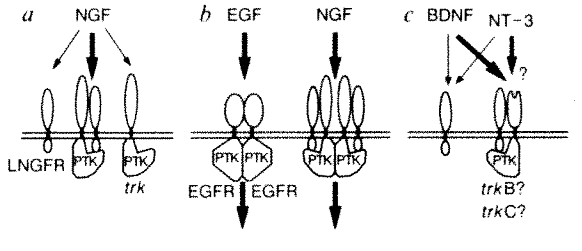
The molecular cloning of a receptor of relative molecular mass 75,000 (M_r 75K) for NGF[4,5] heralded a breakthrough, but excitement was quashed by the finding that this protein exhibited only low-affinity binding to the factor, some 100-fold lower than the dissociation constant K_d of 10^{-11} M linked to such biological effects as neurite outgrowth. Even so, from gene-transfer experiments it seemed that this low-affinity receptor (LNGFR) participated in the high-affinity binding. The search for the 'high-affinity' receptor was bolstered by work from Shooter's laboratory that identified a second NGF-binding protein of 140K in cells displaying high-affinity binding sites. Furthermore, this protein contained phosphotyrosine[6], implying that it might participate in a tyrosine kinase signalling pathway.

Meanwhile, interest in the *trk* family of receptor-like protein-tyrosine kinases was stimulated by their apparent confinement to tissues of neural origin[7,8]. The *trk* oncogene was originally identified by transfection of DNA from a human colon carcinoma[9]: the oncogene consists of a receptor-like protein-tyrosine kinase domain fused to tropomyosin sequences (hence *trk*, tropomyosin receptor kinase). There are at least three cellular family members, *trk*, *trk*B and *trk*C, which are 130–140K proteins each harbouring an extracellular domain and an intracellular tyrosine kinase domain. Despite their receptor-like structure their putative ligands evaded identification. A flurry of papers from three groups now shows that the *trk* ligand is NGF: *trk* kinase activity in PC12 cells is activated minutes after the addition of NGF[10]; p140*trk* can be chemically cross-linked to NGF and is immunologically indistinguishable from the native 140K NGF-binding protein[2,11]; cellular expression of *trk* can confer high-affinity binding for NGF[1,2]; and microinjection of *trk* RNA into *Xenopus* oocytes bestows an NGF-dependent meiotic maturation[12].

So what is the role of the 75K LNGFR? NGF-binding data from the groups of Chao and Parada suggest that, in isolation, *trk* binds NGF with a low affinity similar to that of the 75K LNGFR[11] and that high-affinity binding requires expression of both proteins in the same cellular membrane[1]. Moreover, the 75K protein is clearly implicated in NGF action. In addition to the gene-transfer experiments[1], domain-swapping between the EGF receptor and the LNGFR has demonstrated a role for the transmembrane and cytoplasmic regions of the 75K protein in mediating signal transduction[13]. In this scheme the LNGFR and *trk* proteins are proposed to form a heterodimer that conveys the biological signal (see *a* in the figure). Barbacid's laboratory, by contrast, have presented evidence that *trk* expression alone is sufficient to form high-affinity NGF-binding sites[2]. In addition, when *trk* is introduced into oocytes, a biological response to NGF is conferred, albeit an unnatural one[12].

Which model is correct? Receptor protein-tyrosine kinases, such as the prototypic epidermal growth factor (EGF) receptor, are known to dimerize for signal transmission (see *b* in the figure). Hence, high levels of expression of *trk* may, in the presence of NGF, allow some *trk* homodimer formation and thus generate high-affinity binding sites. Interestingly, the affinity of NGF for *trk* alone, as reported by Barbacid's group, does not appear to be as high as that found for the heterodimer. Given recent evidence suggesting the existence of distinguishable 'high-affinity' binding sites for NGF[14], it will be important to determine the molecular combinations of these receptor subunits required for *biologically* important responses to NGF such as neurite outgrowth.

Just as *trk* is a member of a family of related protein-tyrosine kinase receptors, so NGF is one of at least three related neurotrophic factors, the two others being brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3). The three share structural similarities, but serve as neurotrophins for largely non-overlapping populations of neurons in the central and peripheral nervous systems[15]. An attractive possibility is that the different *trk* proteins may serve as p140 receptor subunits for the different neurotrophins (see *c* in the figure). Interestingly, BDNF and NGF bind to the 75K LNGFR with similar affinities[16], so it is possible that the LNGFR may act as a common receptor subunit for the neurotrophins. Finally, given



In *a*, nerve growth factor (NGF) binds with low affinity to the monomeric p75 NGF receptor (LNGFR) and to *trk*. High-affinity binding is found when both molecules are present[1]. In *b*, the biologically active form of the epidermal growth factor receptor (EGFR) occurs upon dimerization of the protein-tyrosine kinase domains (PTK). Is this the case for the high-affinity NGF receptor? In *c*, two neurotrophic factors related to NGF, brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3)[15], may act through other members of the *trk* PTK family such as *trk*B.

the existence of truncated forms of *trk*B that contain the ligand-binding domain but not the kinase domain[8], it may be that there is a combinatorial heterogeneity to this system, in that the neurotrophin receptors may interact or cross-compete for soluble factors.

'Nerve growth factor' has long been considered something of a misnomer because NGF seems to do nearly everything except stimulate neurons to divide. One striking aspect of the current work is the finding that NGF actually works through a canonical growth-factor receptor, that is, a protein-tyrosine kinase receptor. Moreover, this receptor has oncogenic potential when deregulated. Apparently, the pathways used in signalling cell division in mitogenic cells can be exploited to quite different effect in post-mitotic neurons. The identification of *trk* as a component of the biologically relevant NGF receptor should guarantee rapid progress as the lessons learnt from study of the mitogenic receptor kinases are applied to NGF biology. □

Cliff Ragsdale and Jim Woodgett are at the Ludwig Institute for Cancer Research, 91 Riding House Street, London W1P 8BT, UK.

1. Hempstead, B. L., Martin-Zanca, D., Kaplan, D. R. & Chao, M. V. *Nature* **350**, 678-683 (1991).
2. Klein, R. *et al. Cell* **65**, 189-197 (1991).
3. Levi-Montalcini, R. & Angeletti, P. U. *Physiol. Rev.* **48**, 534-569 (1968).
4. Johnson, D. *et al. Cell* **47**, 545-554 (1986).
5. Radeke, M. J. *et al. Nature* **325**, 593-597 (1987).
6. Meakin, S. O. & Shooter, E. M. *Neuron* **6**, 153-163 (1991).
7. Martin-Zanca, D., Barbacid, M. & Parada, L. F. *Genes Dev.* **4**, 683-694 (1990).
8. Klein, R. *et al. Cell* **61**, 647-656 (1990).
9. Martin-Zanca, D., Hughes, S. H. & Barbacid, M. *Nature* **319**, 743-748 (1986).
10. Kaplan, D. R., Martin-Zanca, D. & Parada, L. F. *Nature* **350**, 158-160 (1991).
11. Kaplan, D. R. *et al. Science* **252**, 554-558 (1991).
12. Nebreda, A. R. *et al. Science* **252**, 558-561 (1991).
13. Yan, H., Schlessinger, J. & Chao, M. V. *Science* **252**, 561-563 (1991).
14. Weskamp, G. & Reichardt, L. F. *Neuron* **6**, 649-663 (1991).
15. Thoenen, H. *Trends Neurosci.* **14**, 165-170 (1991).
16. Rodriguez-Tebar, A., Dechant, G. & Barde, Y.-A. *Neuron* **4**, 487-492 (1990).

FUSION ENERGY

The moment of truth nears

Richard D. Petrasso

THE generation of copious, energetic, charged fusion products (α-particles) and the deposition of their energy within the fusing plasma are pivotal issues for the achievement of fusion energy. With less than nearly perfect α energy input, the plasma cannot sustain the high temperatures (250 million K) needed to maintain deuterium–tritium fusion reactions. In preparation for the first ever experiments on deuterium–tritium plasmas in July 1993 — to occur in the US Tokamak Fusion Test Reactor (TFTR) — a workshop* was held last month to debate the critical issues regarding the plasma physics of α-particles and tritium operations.

Since the first fusion experiments in the early 1950s — conducted mainly with devices called pinches, mirrors and stellarators — all magnetically confined plasmas have been principally composed of weakly fusing, nonradioactive hydrogen isotopes, either hydrogen (mass 1) or deuterium (mass 2). But it was understood that with the eventual attainment of high-quality fusion plasmas, experiments would switch to a mixture of deuterium and tritium (mass 3), the latter being a radioactive β-emitter. Despite the special care required in handling tritium, the overriding reasons for its choice are, first, that the deuterium(d)–tritium(t) reaction

$$\mathrm{d} + \mathrm{t} \rightarrow \alpha(3.5\ \mathrm{MeV}) + \mathrm{n}\ (14.1\ \mathrm{MeV})$$

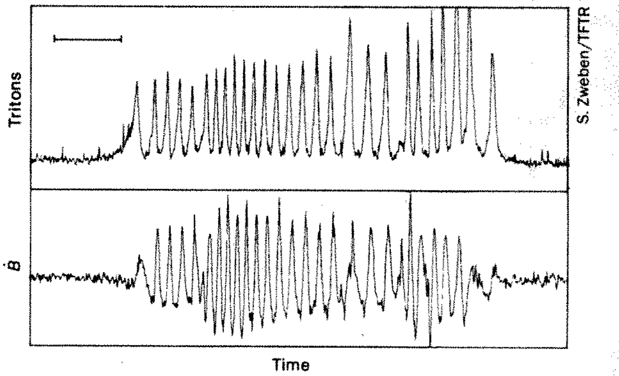
has by far the largest reaction rate and, second, that it occurs at the lowest temperature (n is an energetic neutron, which in principle would be used to power conventional generators, and α is an energetic He^{2+} nucleus).

The large kinetic energy of the α and neutron results from the strong nuclear interaction which converts 0.4 per cent of the deuterium and tritium mass into energy. In contrast ordinary chemical reactions are a million times less energetic, though they too derive their energy by converting mass (as in $mc^2 = E$). But it is precisely because of this million-fold enhancement that, on the one hand, the frightful power of the hydrogen bomb has been realized and, on the other, two generations of physicists and engineers have pursued the goal of fusion energy. One of the principal results of this effort has been the tokamak, a toroidal plasma-containment device in which a large magnetic field (about 50 kilogauss) and plasma current (around 3 megamp) thread the torus, thereby inhibiting the loss of energetic plasma particles.

A figure of merit that represents the quality of any fusion plasma, be it the Sun's core or a tokamak, is $Tn\tau$, where T is the central ion temperature, n the central ion density, and τ the overall energy confinement time (the duration for which the plasma thermal energy can be effectively contained). For a deuterium–tritium plasma to ignite — that is, for the α-energy input to sustain the 250×10^6 K temperature against thermal and radiative losses — $Tn\tau \approx 6 \times 10^{22}$ K cm^{-3} s (the value in the hydrogen-burning solar core, by contrast, is a staggering $5{\times}10^{47}$ K cm^{-3} s: $T = 15{\times}10^6$ K; $n = 10^{26}$ cm^{-3}; and $\tau = 3{\times}10^{14}$ s $=$ 10 million years). The fusion plasma experiments of the 1950s were a factor of 10^7 below this ignition threshold. In the past few years TFTR[1] and JET[2] (Joint European Torus) obtained plasma conditions which, had they been operating with deuterium–tritium under identical conditions, would have momentarily achieved a $Tn\tau$ a factor of 20 and 6 below this threshold. (For JET, $T = 250{\times}10^6$ K; $n = 3.7{\times}10^{13}$ cm^{-3}; and $\tau = 1.1$ s.) Thus although neither tokamak will achieve ignition — nor were they designed to — each can be expected to produce measurable amounts of α power (2 megawatts) and α-particles (3×10^{18} s^{-1}) if using deuterium–tritium fuel. But most of all, several critical issues can be experimentally addressed for the first time.

One such issue is the energy and particle transport in a deuterium–tritium plasma (D. Post, TFTR). Although one might not expect that changing from one hydrogen isotope to another would notably affect the transport, experience teaches otherwise. For example, plasmas of pure deuterium typically run hotter than those of pure hydrogen, and this is partly reflected in a larger value of τ. A corollary is, however, that impurities are also better confined in a deuterium plasma[3]; if inadvertently injected in too large quantities, these could radiatively quench the plasma, as occurred on the record JET discharge[2,4]. Thus it will be important to investigate whether the empirical scaling for the energy confinement, $\tau {\sim} m_i^{1/2}/P^{1/2}$, still holds ($m_i$ is the ion mass and P the total absorbed power), and whether impurities are more likely to reside within the plasma precluding a significant and sustained value of $Tn\tau$.

A second crucial question is what fraction of α-particles will be either kicked out by intrinsic plasma instabilities — such as by sawtooth oscillations and higher-frequency magnetic perturbations (S. Zweben, TFTR; see figure) — or execute a process known as stochastic diffusion whereby they make a rapid random-walk out of the plasma before depositing their energy in the plasma, which takes 0.3 s according to classical calculations



S. Zweben/TFTR

Escaping 1-MeV tritons (upper) resulting from magnetic perturbations $\dot{B}$ (lower) associated with magnetohydrodynamic modes. The energetic tritons are detected by particle detectors just outside the plasma boundary. An important issue is whether these perturbations or others, such as those associated with the α-driven Alfvén mode, will eject a large fraction of the energetic α-particles generated in TFTR deuterium-tritium plasmas. (Scale bar, 1 ms.)

---

*The 1st Workshop on Alpha Physics in the Tokamak Fusion Test Reactor, Princeton, 28–29 March 1991.

(R. Boivin, TFTR). In principle, each of these effects has been observed at TFTR through energetic protons (p) and tritons (t) created in the fusion reaction

$$d + d \rightarrow p\ (3\ \text{MeV}) + t\ (1.0\ \text{MeV})$$

And in closely related work at JET (F. Marcus), 14.7-MeV protons from deuterium–helium-3 reactions were poorly confined with a 1.5 MA plasma current, but well confined with a 4.5 MA current unless sawtooth oscillations occurred. For deuterium–tritium plasmas, the crux of the issue will be quantitative: what fraction of the α-particles will be so affected, and how often?

A conceptually related issue is whether the large population of α-particles will themselves generate and drive formerly benign or unknown collective oscillations in the plasma that would again result in their 'self ejection'. Of the class of such instabilities that physicists theorize, none was more widely discussed than the α-driven Alfvén mode[5-7]. This instability should occur if a sufficient number of α-particles have, along the direction of the magnetic field, a component of velocity greater than a critical velocity called the Alfvén velocity, V_A (D. Sigmar, MIT). (The tokamak's confining magnetic field includes a toroidal component and a smaller poloidal field created by the plasma current.)

The Alfvén velocity, which depends on the field strength (B) and central ion density ($V_A = B/\sqrt{4\pi m_i n_i}$), is around 0.8×10^9 cm s^{-1} (with a field of 50 kilogauss and central ion density of 6×10^{13} cm^{-3}). The initial velocity of the 3.5-MeV α-particles is 1.3×10^9 cm s^{-1}, and about 40 per cent will have a 'parallel' velocity component exceeding the Alfvén velocity.

But it is also becoming apparent that two other conditions need to be satisfied if the Alfvén mode is to be excited. First, the number density of α-particles at the centre must not be less than about 0.3 per cent of the electron density. And second, the α-particles' gas-kinetic pressure must exceed 0.003 times the magnetic pressure they experience ($B^2/8\pi$, about 100 atmospheres).

Simulations of TFTR deuterium–tritium plasmas (R. Bundy) find all three thresholds likely to be exceeded. But recent "α-simulation experiments" (K. Wong) on TFTR using energetic neutral beams and deuterium plasmas indicate that the pressure threshold may be larger by about an order of magnitude[7]. If so, then TFTR deuterium–tritium plasmas may be spared this particular α-particle loss channel.

The best method of injecting tritium into TFTR is also of pressing importance. The most frequently discussed methods are injecting it either with high-power heating beams, or firing in tiny frozen tritium pellets. Among other compelling arguments for using pellets (each less than 0.01 g in mass, equivalent to 100 curies of radioactivity), one is that it will tend to place the tritium mostly on the tokamak axis where it can be more effectively burned (D. Mikkelsen, TFTR). Another not inconsequential benefit is that pellet injection reduces by an order of magnitude the total amount of tritrium injected into the machine (D. Meade, TFTR).

Making maximum use of the limited number of deuterium–tritium discharges is another concern (J. Strachan, TFTR); only 1,000 or so are feasible, whereas TFTR has already had about 50,000 hydrogen, deuterium and helium discharges. For example, should new diagnostic methods be developed that in principle could, with some spatial and temporal resolution, measure aspects of the α-particles' energy distribution? Although highly desirable, the means to measure these parameters are at present untested, although serious efforts to do just that are underway (P. Woskov, MIT). And even if not immediately applicable to TFTR, they might prove invaluable for the next generation of experiments.

An approach that addresses the immediate needs of TFTR is to place greater emphasis on proven methods that could measure the escaping α-particles (as with tritons as shown in the figure). Largely in combination with the α-particle birth profile (from 14.1-MeV neutron imaging), such measurements should reveal a clear picture of the overall α-particle transport.

Furthermore, it will be essential to establish experimentally for each diagnostic technique — such as those used to measure the ion-temperature profile or the plasma impurity content — its ability to operate reliably in the intense neutron and γ-ray fluxes resulting from the fusion neutrons. None of the tokamak diagnostic techniques has yet operated in such a severe environment, so that there is concern that, despite careful shielding and other precautions, some measurements might give false or unintelligible signals owing to the background radiations.

Finally, assuming that most of the α-particles do deposit their energy in the plasma, what is to be done with the thermalized He^{2+} 'ash' which would certainly dilute the deuterium–tritium fuel and so extinguish high-temperature ignition? One proposed solution (G. Miley, Univ. Illinois) is that suitable plasma instabilities should be periodically triggered so as to expel the ash. However, from this and previous considerations it is clear that a fine line has to be walked: on the one hand energetic α-particles must deposit their energy in the plasma; on the other, having done so, they must be transported out. TFTR should give us our very first glimpse of this delicate balance. □

Richard D. Petrasso is at the Plasma Fusion Center, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA.

---

1. Meade, D. *et al.* IAEA 1990, W. D. C. (Proc. to be published).
2. Keilhacker, M. *et al. Phys. Fluids* **2**, 1291 (1990).
3. Marmar, E. *et al. Nucl. Fus.* **22**, 1567 (1982).
4. Petrasso, R. D. *Nature* **343**, 21–22 (1990).
5. Fu, G. Y. & Van Dam, J. *Phys. Fluids* B**1**, 1949 (1989).
6. Sigmar, D. S., Hsu, C., White, R. & Cheng, C. Z. MIT Plasma Fusion Center, Rep. No. PFC/JA-89-58 (1989).
7. Wong, K. L. *et al. Phys. Rev. Lett.* **66**, 1874–1877 (1991).

DAEDALUS

Dead Heat

**Last week Daedalus proposed a nove
sound-absorber. It is a rubber plasticize
with high-pressure gas, so that th
contractile tension in the rubber matrix i
exactly balanced by the pressure of th
occluded gas. Over a wide density-rang
it has no preferred volume and zer
elasticity. So sound traverses it with zer
velocity, and never gets anywhere.**

**Both as a perfect sound-insulator, and a
a completely 'dead' vibration-damper, th
new polymer/gas composite will fin
innumerable applications. But Daedalu
now points out that heat is also a form o
sound; it is propagated by therma
phonons. A gas-swollen polymer balance
to have zero phonon-velocity should be
perfect heat-insulator.**

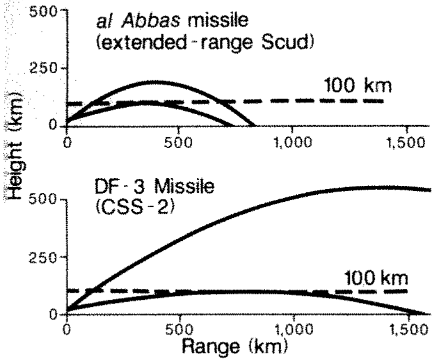
**At last the bulkiness and inconvenienc
of conventional lagging will be overcome
Even in thin sheet form, DREADCO's ne
"Dead Heat" polymer will totally insulat
refrigerators and ovens, hot-water tank
and pipes, walls and windows, floors an
ceilings, and all other surfaces throug
which heat leaks wastefully away. Office
will be fully heated by their content of sta
and computers, frozen foods and ho
drinks will be sold on open shelves in Dea
Heat cartons, and a new market may ope
up for well-wrapped parcels of industri
waste heat. And on the human scale, th
unstated modern tension between fashio
and domestic heating will at last b
resolved. Respectable victorians, wit
many layers of woollen underwear, coul
tolerate houses at 10 °C. We demand
wasteful 20 °C in order to show off ou
bodily elegance under a minimum thick
ness of tight-fitting clothing. But tailored i
DREADCO's Dead Heat fabrics, even th
thinnest jeans, most figure-hugging gown
or negligible negligees will be as warm an
comfortable as arctic survival-gear.**

**They may feel a bit like it, too. The ne
fabrics, with their complete absorption
movement and vibration, will insulate th
wearer from the innumerable sma
touches and contacts which make u
kinetic awareness. The resulting sense
isolation and numbness could be qui
disturbing, especially in clothes like glove
boots and underpants. The material ma
have to be slightly unbalanced, giving it
slight vibration-velocity.**

**Such a material would also make a
intriguing thermal delay-line. A hig
temperature applied to one side of it wou
emerge from the other side maybe hou
later. This could be very useful.
housewall of the product, for exampl
could be formulated to have a twelve-ho
delay. More economical even than a tot
thermal insulator, it would retard t
warmth of the day till night-time, whi
allowing the cool of midnight into t
premises around noon.** David Jone

Underflying Brilliant Pebbles

SIR — Following the use of the Patriot missile in the Gulf War, the US Strategic Defense Initiative Office (SDIO) claims that its proposed space-based antimissile system Brilliant Pebbles could be used to destroy theatre ballistic missiles such as the extended-range Scud (the *al Abbas*), but its claim ignores a simple and effective countermeasure. SDIO has acknowledged that Brilliant Pebbles can intercept missiles only above about 100 km. Accordingly, if a missile's trajectory is adjusted so that it stays below this altitude throughout its flight, the missile becomes immune to Brilliant Pebbles. Our calculations show that flying theatre missiles on such depressed trajectories is not technically demanding; missiles with ranges considerably greater than those used by Iraq can underfly Brilliant Pebbles on trajectories with apogees of 100 km or less.



Minimum-energy maximum-range trajectories, and depressed trajectories with apogees of 100 km for the *al Abbas* and DF-3 missiles. The *al Abbas* has a maximum range of 830 km with a 250-kg warhead, and reaches an apogee of 190 km; on the depressed trajectory, it still has a range of 720 km. With a 2,000-kg payload, the DF-3 has a maximum range of 2,780 km with an apogee of 550 km; on the depressed trajectory, its range would be 1,550 km.

The Brilliant Pebbles altitude limit results from the high atmospheric densities at low altitudes; flying through regions of dense atmosphere at high speeds would blind the interceptors by heating their sensors. On 12 February 1991, SDIO director Henry Cooper stated that Brilliant Pebbles could not attack targets below roughly 60 miles (100 km). Lowering this altitude limit is difficult because of the exponential increase in atmospheric density with decreasing altitude. For example, in going from an altitude of 100 to 80 km, the density and resulting heating increase by a factor of 40.

To determine whether a missile could be flown on a depressed trajectory, we considered whether doing so would lead to unacceptable increases of stress ('loading') on the missile or heating on the re-entry vehicle and how it would affect the range and accuracy. We calculated the missile trajectories by numerically integrating the equations of motion[1], including forces due to gravity and atmospheric drag (lift was ignored). We compared the loading and heating for the depressed trajectory to that of the maximum-range minimum-energy trajectory for the same payload. The loading is roughly proportional to ρv^2, where ρ is the atmospheric density and v is the missile velocity; for the coefficient, we used measured values for the V2 missile[2]. The aerodynamic heating on re-entry is roughly proportional to ρv^3, and was calculated using equations for laminar and turbulent hypersonic flow (assuming a boundary layer transition at 30–35 km)[3].

To be specific, we considered two theatre-range missiles deployed in the Middle East: the 800-km Iraqi *al Abbas* and the Chinese-built 2,800-km DF-3 (CSS-2) missile deployed by Saudi Arabia. For the *al Abbas*, we assumed a total mass of 6,900 kg, fuel fraction of 80%, payload mass of 250 kg, specific impulse of 245 s, burntime of 115 s and booster diameter of 0.9 m (refs 4, 5 and S. Fetter, personal communication). For the DF-3, these parameters were taken to be 65,500 kg, 93.7%, 2,000 kg, 241 s, 142 s and 2.25 m (ref. 6). The weight-to-drag ratio for the DF-3 re-entry vehicle was taken as 48,000 N m^{-2}.

Calculations were done for depressed trajectories with apogees of 100 and 80 km. The loading was found to increase by 5–7% for the *al Abbas*, and 10–11% for the DF-3, which should be within the tolerances of both missiles. The heating was found to increase by 25–30% for the *al Abbas*, and 20–25% for the DF-3. This increase should not present a problem for the relatively crude *al Abbas*, for which the entire missile acts as the re-entry vehicle. If the increase in heat absorbed by the DF-3 RV exceeds its design tolerances, the heat shielding could be increased slightly, or the heating could be reduced to that of the standard trajectory by reducing the payload by 25%.

Flying missiles on depressed trajectories decreases their range; the percentage change increases with range (see figure). However, missiles with ranges of less than 1,500 km are sufficient to threaten most targets in the Middle East.

Our estimates suggest that the accuracy of these theatre ballistic missiles, which are very inaccurate, would not change significantly. Although errors due to atmospheric effects would increase on low-altitude trajectories, the dominant errors for these missiles are caused by the guidance system and would not be substantially affected. The inaccuracy of the *al Abbas*, estimated at 3–5 km (ref. 7), is not expected to change. The DF-3 inaccuracy of roughly 2.4 km (ref. 8) might increase by a factor of 2 to 3. As the original inaccuracy is so large that the only targets would be cities, such an increase would not remove its utility as a terror weapon.

Thus, theatre missiles like the *al Abbas* and DF-3 missiles would be able to fly on low-apogee depressed trajectories, thereby rendering them invulnerable to space-based defences.

DAVID C. WRIGHT
Federation of American Scientists,
307 Massachusetts Avenue, NE,
Washington DC 20002, USA
LISBETH GRONLUND
Center for International Security Studies,
University of Maryland,
7100 Baltimore Boulevard,
Suite 400, College Park,
Maryland 20740, USA

1. Regan F. J. *Reentry Vehicle Dynamics* 287 (American Institute of Aeronautics and Astronautics, New York, 1984).
2. Sutton, G. P. *Rocket Propulsion Elements* (3rd ed) 199 (Wiley, New York, 1965).
3. Anderson, J. D. *Hypersonic and High Temperature Gas Dynamics* 288–291 (McGraw-Hill, New York, 1989).
4. Zaloga, S. *International Defense Review* **21**, 1423 (1988).
5. Cochran, T. B., Arkin, W. A., Norris, R. S. & Sands, J. I. *Soviet Nuclear Weapons* 220, (Ballinger, Harper and Row, Cambridge, Massachusetts, 1989).
6. Huang, Z. & Ren, X. *Space Technology* **8**, 371 (1988).
7. *Jane's Defence Weekly* **15**, 103 (1991).
8. Shuey, R. D. *et al. Missile Proliferation: Survey of Emerging Missile Forces* 65 (Congressional Research Service, Washington, 1988).

Is C_{60} stiffer than diamond?

SIR — The availability of single crystals of C_{60} (ref. 1) should allow measurements of the stiffness of these molecules. By using a simple elasticity argument, we estimate the bulk modulus of individual C_{60} molecules to be 843 GPa, which is greater than that of diamond (441 GPa). We expect that the modulus of single crystals of C_{60} will reach 642 GPa at modest pressures and that crystalline C_{60} will then be stiffer than diamond at the same pressure.

Consider a single crystal of graphite with equal orthogonal tensile (or compressive) stress components applied to areas normal to the basal plane. Let x_3 be the direction along the c axis and x_1 and x_2 lie in the basal plane. The stress state is

$$\begin{bmatrix} \sigma_1 & & \\ & \sigma_2 & \\ & & 0 \end{bmatrix} = \begin{bmatrix} \sigma & & \\ & \sigma & \\ & & 0 \end{bmatrix}$$

Hence the longitudinal strains ε_1 and ε_2 are given by

$$\varepsilon_1 = \varepsilon_2 = S_{11}\sigma_1 + S_{12}\sigma_2 = (S_{11}+S_{12})\sigma$$

where the S_{ij} are elastic compliances. The dilation ΔA of the basal area A is

$$\frac{\Delta A}{A} = \varepsilon_1 + \varepsilon_2 = 2(S_{11} + S_{12})\sigma$$

If h is the height of a graphite basal plane (one half of the lattice spacing c because of the ABAB stacking sequence) then

$$\frac{\Delta A}{A}h = 2(S_{11}+S_{12})\sigma h = 2(S_{11}+S_{12})\gamma \qquad (1)$$

where γ is the membrane tension for a single graphite layer. (This behaves as a surface tension). If we now assume that γ is curvature-independent and that the molecules are spherical with radius R, the hydrostatic tension P_t is given by the Laplace equation $P_t=2\gamma/R$. If we treat the C_{60} molecule more correctly as a truncated icosahedron rather than a sphere, the right-hand side of this equation must be multiplied by 1.0881. Substituting for γ in equation (1),

$$P_t = \frac{h}{R}\ \frac{1.088}{(S_{11}+S_{12})}\ \frac{\Delta A}{A} \qquad (2)$$

or, in terms of the volume V,

$$P_t = \left[\frac{2}{3}\ \frac{h}{R}\ \frac{1.088}{(S_{11}+S_{12})}\right] \frac{\Delta V}{V} \qquad (3)$$

and so we obtain for the bulk modulus at zero pressure,

$$B_0 = \frac{2}{3}\ \frac{1.088}{(S_{11}+S_{12})}\ \frac{h}{R} \qquad (4)$$

As this argument is based on linear elasticity, hydrostatic pressure (instead of tension) would give the same result. As the molecule is treated here as an elastic continuum, under hydrostatic pressure the pressure everywhere within the molecule is the same: the pressure at the molecular radius R_{AC} is the same as the external pressure P. Hence we do the energy balance leading to equation (4) at the surface of the truncated icosahedron defined by R_{AC}. For a quantitative estimate of B_0 we use the experimental quantities[2] $S_{11} = 0.00098\ \text{GPa}^{-1}$, $S_{12} = -0.00016\ \text{GPa}^{-1}$ and $h = c/2 = 3.354$ Å (ref. 3). For the C_{60} ball, $R_{AC} = 3.52$ Å (this is the distance from the centre of the ball to the centre of the atoms on the ball's surface (T. Siegrist, personal communication)). This yields $B_0 = 843$ GPa, which is larger than the modulus of 441 GPa found experimentally for diamond[4].

The molecular radius obtained from the lattice parameter of the closest-packed f.c.c. crystal is $R_{CP} = 5.02$ Å (ref. 1). When C_{60} balls are placed on a f.c.c. lattice, they interact essentially via van der Waals attractive forces (as do Ne, Ar and Kr) and initially the crystal would be compliant (the bulk modulus would be relatively small). When the pressure is increased to the point where the hard spheres touch, however, the bulk modulus of the crystal will become similar to that for a single molecule: the filling factor for balls in an f.c.c. crystal is 0.74, so that using the volume-fraction rule we obtain $B = 843 \times 0.74 = 624$ GPa for such (presumably modest) pressures.

The hardness of materials is related to their modulus[5]. It is thus possible that at pressures of about 20 GPa or more (where the soft-sphere repulsion is overcome), f.c.c. C_{60} crystals will be harder than diamond, and even harder than the hypothetical C_3N_4 compound suggested by Cohen[6].

Rodney S. Ruoff

IBM Research Division,
T. J. Watson Research Center,
Yorktown Heights,
New York 10598, USA

Arthur L. Ruoff

Department of Materials Science
and Engineering,
Cornell University,
Ithaca, New York 14853, USA

---

1. Kraetschmer, W., Lamb, L. D., Fostiropoulos, K. & Huffman, D. *Nature* **347**, 354–358 (1990).
2. Kelly, B. T. *Physics of Graphite* **74**, (Applied Science Publishers, Englewood, New Jersey, 1981).
3. Barrett, C. S. & Massalski, T. B. *Structure of Metals* **627**, (McGraw-Hill Book Co., New York, 1966).
4. McSkimin, H. J. & Bond, W. L. *Phys. Rev.* **105**, 116–121 (1957).
5. Westbrook, J. H. & Conrad, H. *The Science of Hardness Testing and its Research Applications* (American Society for Metals, Metals Park, Ohio, 1973).
6. Liu, A. Y. & Cohen, M. L. *Science* **245**, 841–842 (1989).

Plastid genes and parasitic plants

Sir — Howe and Smith[1] have provided one explanation for the retention of a plastid genome in achlorophyllous *Epifagus virginiana*, as reported by dePamphilis and Palmer[2]. But porphyrin biosynthesis is not the only significant metabolic activity restricted to plastids; the biosynthetic pathways for glutamate, lysine, threonine, methionine, isoleucine, leucine, valine, tryptophan, phenylalanine, tyrosine, arginine, cysteine, serine and glycine are wholly or partly located within the plastid[3]. Thus the synthesis of most of the protein amino acids, and all the metabolites derived from them, is dependent on intact and functional plastids. Other metabolic pathways, not necessarily linked to photosynthesis, are also localized in plastids.

Even in a plant that has lost one aspect of plastid metabolism, namely photosynthetic carbon fixation, there would be little advantage in losing all plastid functions. The genes for all the biosynthetic enzymes for the pathways mentioned are encoded in the nucleus, and the polypeptides produced from these genes have leader sequences directing them to plastids. It is thus hard to envisage any evolutionary process in which all these enzymes could be redirected (simultaneously?) to other parts of the cell, and their functions integrated there.

The plastid is an essential component of the plant cell — the chloroplast is merely a specialized plastid restricted to a few cell types and tissues. So the retention of a plastid genome in *E. virginiana* is not remarkable, although its absence most surely would be.

Roger M. Wallsgrove

Soils and Crop Sciences Division,
Rothamsted Experimental Station,
Harpenden,
Hertfordshire AL5 2JQ, UK

---

1. Howe, C. J. & Smith, A.G. *Nature* **349**, 109 (1991).
2. dePamphilis, C. W. & Palmer, J. D. *Nature* **348**, 337–339 (1990).
3. Lea, P. J., Wallsgrove, R. M. & Miflin, B. J. in *Chemistry and Biochemistry of the Amino Acids* (ed. Barrett, G.C.) 197–226 (Chapman & Hall, London & New York, 1985).

Muscle damage in mdx mice

Sir — Menke and Jockusch reported[1] th
hypo-osmotic shock exposes a reduce
stability of dystrophin-deficient mdx mous
muscle. However, this is not apparent wit
experimental models of damage to md
mouse muscle induced by contractile activ
ity[2] or as an enhanced damage response i
people with Duchenne muscular dystroph
who exercise[3].

The isolated, intact mature muscle-fibr
studies by Menke and Jockusch were vi
tually free of extracellular matrix as a resu
of the collagenase treatment used in the iso
lation procedure. Surrounding muscle cell
and pericellular fibrous tissue may well exe
a restraining effect on muscle cells *in viv*
preventing the substantial distortions of th
membrane and hypercontraction appare
in hypo-osmotic shock to isolated muscl
fibres. Further, the nature of the mechanis
of cell death is unknown in hypo-osmoti
shock, but the stress on the membrane pre
sumably involves a substantial increase i
internal pressure in the myofibres as extru
sions (blebs) on the cell membrane ar
formed, a type of stress not presented to th
muscle fibre in the models of contractil
activity induced by damage.

Interestingly, the progression of the dam
age to the isolated fibres described by Menk
and Jockusch is not unique to hypo-osmoti
shock, but is qualitatively similar to th
which occurs in isolated fibres under variou
forms of stress, including trauma and de
liberate elevation of intracellular calciu
with the calcium ionophore[4]. It therefo
seems necessary to determine whether th
decreased stability of mdx muscle found b
Menke and Jockusch is a consequence of th
hypo-osmotic shock or of the nature of th
model system used. Although the results
Menke and Jockusch's report are interes
ing and potentially important, the 'fragi
membrane' theory is by no means prove
for an abnormality such as increased fragili
to be important in the pathogenesis
Duchenne and Becker muscular dystroph
it must be apparent under physiologic
conditions.

M. J. Jacks
A. McArd
R. H. T. Edwar

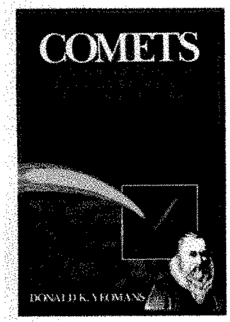
Muscle Research Centre,
Department of Medicine,
University of Liverpool,
PO Box 147,
Liverpool L69 3BX, UK

D. A. Jon

Department of Medicine,
University College,
London WC1E 6BT, UK

---

1. Menke A. & Jockusch H. *Nature* **349**, 69–71 (1991).
2. Jackson M. J., McArdle A. & Edwards R. H. T. *J. Neurol Sci.* (suppl.), 239 (1990).
3. Jackson M. J. *et al. Muscle & Nerve* **10**, 15–21 (1987)
4. Zuurveld J. G. E. M., Veerkamp J. H. and Wirtz P. *Muscl* *Nerve* **8**, 750–759 (1985).



WILEY

COMETS

A Chronological History of
Observation, Science,
Myth and Folklore

By Donald K. Yeomans,
Research Astronomer, NASA,
Jet Propulsion Laboratory, USA

"It is a superb book – a veritable mine of information for both expert and beginner."
– DR. PATRICK MOORE

- From the "specialist's specialist" – a monumental achievement destined to be the standard reference on comets
- Clearly explains the basic astronomy and physics of comets
- Features a comprehensive catalogue of all cometary apparitions up until 1700 – a feature found in no other book
- Covers the full range of comet fact and fiction, science and sorcery, from China to the Americas, the Stone Age to recent observations
- 496 pages with over 100 illustrations and diagrams
- Appendix of comet data and 46 pages of bibliography

471 61011 9 Published 18th April £24.95 Hardback

*Published by John Wiley & Sons Ltd, Baffins Lane, Chichester, West Sussex
PO19 1UD Tel: (0243) 770318 Fax: (0243) 527944. Order from your bookseller
r direct from the Publisher (please add £1.95 p&p when ordering from the
ublisher).*

Don Yeomans will be appearing on BBC TV's "Sky at Night"
Programme with Patrick Moore on 28th April.

ader Service No.2

GUIDE TO AUTHORS

se follow these guidelines so that your manuscript may be
ed expeditiously.
ure is an international journal covering all the sciences. Contri-
s should therefore bear in mind those readers who work in other
and those for whom English is a second language, and write
y and simply, avoiding unnecessary technical terminology.
in the journal is limited, making competition for publication
. Brevity is highly valued. One printed page of *Nature*, without
uptions of the text, contains about 1,300 words.
nuscripts are selected for publication according to editorial
ment of their suitability and reports from independent referees.
can be sent to London or Washington and should be addressed
Editor. Manuscripts may be dealt with in either office, depend-
the subject matter, and will where necessary be sent between
s by overnight courier. All manuscripts are acknowledged on
t but fewer than half are sent for review. Those that are not
ved are returned as rapidly as possible so that they may be
tted elsewhere without delay. Contributors may suggest
vers; limited requests for the exclusion of specific reviewers are
y heeded. Manuscripts are usually sent to two or three review-
ho are chosen for their expertise rather than their geographical
on. Manuscripts accepted for publication are typeset from the
on office.
ure requests authors to deposit sequence and crystallographic
n the databases that exist for this purpose, and to mention
bility of these data.
ce a manuscript is accepted for publication, contributors will
e galley proofs in about 4 weeks. *Nature*'s staff will edit
scripts with a view to brevity and clarity, so contributors should
their proofs carefully. Manuscripts are generally published 2–3
s after receipt of corrected proofs. *Nature* does not exact page
es. Contributors receive a reprint order form with their proofs;
t orders are processed after the manuscript is published and
ent received.

Categories of paper

w articles survey recent developments in a field. Most are
issioned, but suggestions are welcome in the form of a one-page
sis addressed to the Reviews Coordinator. Length is negotiable
ance.
s are research reports whose conclusions are of general
st and which are sufficiently rounded to be a substantial advance
erstanding. They should not have more than 3,000 words of text
ncluding figure legends) or more than six display items (figures
bles) and should not occupy more than five pages of *Nature*.
icles start with a heading of 50–80 words written to advertise
content in general terms, to which editors will pay particular
ion. The heading does not usually contain numbers, abbre-
ns or measurements. The introduction to the study is contained
first two or three paragraphs of the article, which also briefly
arize its results and implications. Articles have fewer than 50
ences and may contain a few subheadings of two or three words.
s are short reports of outstanding novel findings whose impli-
s are general and important enough to be of interest to those
e the field. Letters should have 1,000 or fewer words of text and
r fewer display items. The first paragraph describes, in not more
150 words and without the use of abbreviations, the background,
ale and chief conclusions of the study for the particular benefit
n-specialist readers. Letters do not have subheadings and
in fewer than 30 references.
mentary articles deal with issues in, or arising from, research
are also of interest to readers outside research. Some are
nissioned but suggestions can be made to the commentary editor
e form of a one-page synopsis. Commentaries are normally
een one and four pages of *Nature*.
and Views articles inform non-specialist readers about new
tific advances, sometimes in the form of a conference report.
are commissioned but proposals can be made in advance to the
News and Views editor.

Scientific Correspondence is for discussion of topical scientific matters, including those published in *Nature*, and for miscellaneous contributions. Priority is given to letters of fewer than 500 words and 5 references.

Preparation of manuscripts

All manuscripts should be typed, double-spaced, on one side of the paper only. An original and three copies are required, each accompanied by artwork. If photographs are included, four sets of originals are required; for line drawings, one set of originals and three good-quality photocopies are acceptable. Reference lists, figure legends and tables should all be on separate sheets, all of which should be double-spaced and numbered. Relevant manuscripts in press or submitted for publication elsewhere should be included with each copy of a submitted manuscript, and clearly marked as such. Revised and resubmitted manuscripts should also be clearly marked as such and labelled with their manuscript numbers.

Titles say what the paper is about with the minimum of technical terminology and in fewer than 80 characters in the case of Articles and Letters. Active verbs, numerical values, abbreviations and punctuation are to be avoided. Titles should contain one or two key words for indexing purposes.

Artwork should be marked individually and clearly with the author's name and, when known, the manuscript number. Ideally, no figure should be larger than 28 by 22 cm. Figures with several parts are to be avoided and are permitted only if the parts are closely related, either experimentally or logically. Unlettered originals of photographs should be provided. Original artwork is returned when a manuscript cannot be published.

Protein/nucleotide sequences should ideally be in the three-letter and not the single-letter code for amino acids. One column width of *Nature* can accommodate 20 amino acids or 60 base pairs. Numbering of sequences should be in the left-hand margin only, with a single space between rows.

Suggestions for cover illustrations, with captions and labelled with the manuscript number, are welcome.

Colour artwork. A charge of £500 per page is made as a contribution towards the cost of reproducing colour figures. Inability to pay these costs will not prevent the publication of essential colour figures if the circumstances are explained. Proofs of colour artwork may be sent to contributors under separate cover from their galley proofs.

Figure legends should not exceed 300 words and ideally should be shorter. The figure is described first, then, briefly, the method. Reference to a method published elsewhere is preferable to a full description. Methods are not described in the text.

References are numbered sequentially as they appear in the text, followed by those in tables and finally by those in figure legends. Only papers published or in the press are numbered and included in the reference list. All other forms of reference, including unrefereed abstracts, should be cited in the text as a personal communication, manuscript submitted or in preparation. Text is not included in reference lists. References are abbreviated according to the *World List of Scientific Periodicals* (Butterworths, London, 1963–65). The first and last page numbers are included; reference to books should include publisher, place and date.

Abbreviations, symbols, units and greek letters should be identified the first time they are used. Acronyms should be avoided whenever possible and, if used, defined. Footnotes are not used except for changed addresses.

Acknowledgements are brief; grant and contribution numbers are not allowed.

Submission. Manuscripts can be sent to the Editor at 4 Little Essex Street, London WC2R 3LF, UK or at 1137 National Press Building, Washington, DC 20045, USA. Manuscripts or proofs sent by air courier to London should be declared as 'manuscripts' and 'value $5' to prevent the imposition of import duty and value-added tax (VAT). □

RETROVIRAL PROTEASES

Control of Maturation and Morphogenesis

Laurence Pearl, Lecturer in Molecular Biology,
University College, London

This volume describes the state-of-the-art of our understanding of the structure and function of retroviral proteases, and their substrates, the viral polyproteins, described by the leading workers in the field. The contributions range from detailed biochemical and structural characterisation of several retroviral proteases including that from HIV-1, through the analysis of the proteolytic processing of the viral polyproteins, to the structure of the viral capsids formed by the action of the protease, and is essential reading for anyone interested in the molecular biology of AIDS.

Contents: Introduction • *L.Pearl* Characterisation and Localisation of HIV Protease Activity; *K.von der Helm* Characterisation of the HIV Protease as a Molecular Target for the Treatment of AIDS and Related Disorders; *C.Debouck* Analysis of Recombinant HIV Pol Gene Products; *K.Moelling* Substrate Specificity and Inhibitors of Retroviral Proteases; *Y.Yoshinaka* HIV Pol Expression via a Ribosomal Frameshift; *A.Kingsman* Reverse Transcriptase Activity of HIV-1 gag-pol Fusion Protein; *C.Peng* Processing gag and pol Gene Products of HIV; *M.Nai* Dimerisation of HIV Protease Investigated by Gel-permeation Chromatography; *O.Singh* Three Dimensional Structure and Evolution of Retroviral Proteases; *T.Blundell* X-Ray Analysis of HIV-1 Protease and its Complexes with Inhibitors; *M.Miller* Assembly of the HIV Pre-Core Particle in Insect Cells Expressing P55 Antigen; *I.Jones* Morphogenesis of Retroviruses: A Complex Role for the Matrix (MA) Protein in Assembly, Stability and Intracellular Transport of Capsid Proteins; *E.Hunter* The Action of Retroviral Protease in Various Phases of Virus Replication; *S.Oroszlan* Terminal Stages of Retrovirus Morphogenesis; *R.Luftig* Electron Microscope Tomography of HIV; *S.Hoglund* Structure of HIV Particles; *H.Gelderblom* • Index

Published: 1990 Pages: 192pp Size: 234x156mm
Binding: Paperback £27.50 0–333–53612–6

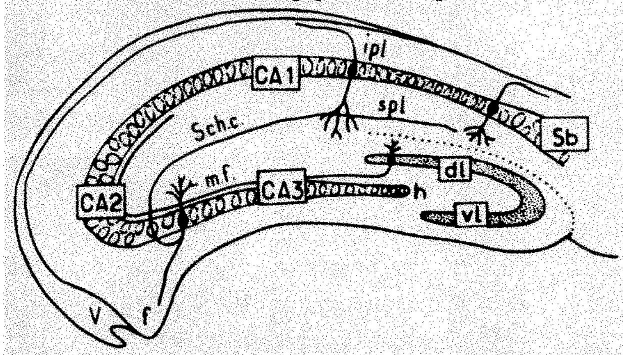
Available from your local bookseller or from
Dionne Stocking, Macmillan Press, Houndmills,
Basingstoke, Hants RG21 2XS.

Update your Springer Library

Books to catch your interest!

K. Y. Reznikov

Cell Proliferation and Cytogenesis in the Mouse Hippocampus



1991. Approx. 80 pp. 30 figs. 7 tabs. (Advances in Anatomy, Embryology and Cell Biology, Vol. 122)
Softcover DM 98,- ISBN 3-540-53689-2

Contents: Hippocampal Formation in the Mouse and Rat. - Materials and Methods. - Cell Proliferation and Cell Death in the Developing Hippocampus of a Mouse. - Postnatal Production and Death of Cells in the Mouse Dentate Gyrus. - Neurogenesis in the Hippocampus and Neocortex as the Embryonic Basis for Brain Module Formation. - Summary. - Subject Index.

R. Seifert, G. Schultz

The Superoxide-Forming NADPH Oxidase of Phagocytes: An Enzyme System Regulated by Multiple Mechanisms

1991. VI, 274 pp. 18 tabs. (Reviews of Physiology, Biochemistry and Pharmacology, Vol. 117)
Hardcover DM 148,- ISBN 3-540-53663-9

This book reviews the literature on the regulation of NADPH oxidase, which is controversial in many areas, and refers specifically to relevant publications from the past 4 years. Main topics are catalytical properties, co-factor requirements and structural components, activation, inhibition, and pathology of NADPH oxidase, and reconstitution and regulation of NADPH oxidase activity in cell-free systems.

D. J. Ramsay, D. A. Booth (Eds.)

Thirst

Physiological and Psychological Aspects

1991. Approx. 545 pp. 91 figs.
(ILSI Human Nutrition Reviews)
Hardcover DM 245,- ISBN 3-540-19641-2

The subject of thirst and drinking behavior are uniquely discussed in this book. For the first time both the physiological and the psychological aspects of water and beverage consumption are examined in one volume. The many recent developments concerning how a lack of water is signalled physiologically and processed neurally to affect drinking behavior are critically surveyed.

L. Fishbein (Ed.)

Biological Effects of Dietary Restriction

1991. Approx. 335 pp. 80 figs. 99 tabs.
(ILSI Monographs) Hardcover DM 220,-
ISBN 3-540-52294-8

How does dietary restriction affect the physiological and biochemical state of laboratory animals? How will the present conclusions affect further research? What are the implications for human health and safety assessment? These are the main questions asked in this book by leading international researchers.

For further information, please contact your bookseller, use the Nature Reader Service Card, or write directly to Springer-Verlag.
Prices are subject to change without notice.

Springer-Verlag
Berlin
Heidelberg
New York
London
Paris
Tokyo
Hong Kong
Barcelona
Budapest

☐ Heidelberger Platz 3, W-1000 Berlin 33, F. R. Germany
☐ 175 Fifth Ave., New York, NY 10010, USA
☐ 8 Alexandra Rd., London SW19 7JZ, England
☐ 26, rue des Carmes, F-75005 Paris, France ☐ 37-3, Hongo 3-chome, Bunkyo-ku, Tokyo 113, Japan
☐ Room 701, Mirror Tower, 61 Mody Road, Tsimshatsui, Kowloon, Hong Kong ☐ Avinguda Diagonal, 468-4°C, E-08006 Barcelona, Spain

tm.40.109/ SF

Reader Service No.6

They also serve

William H. Press

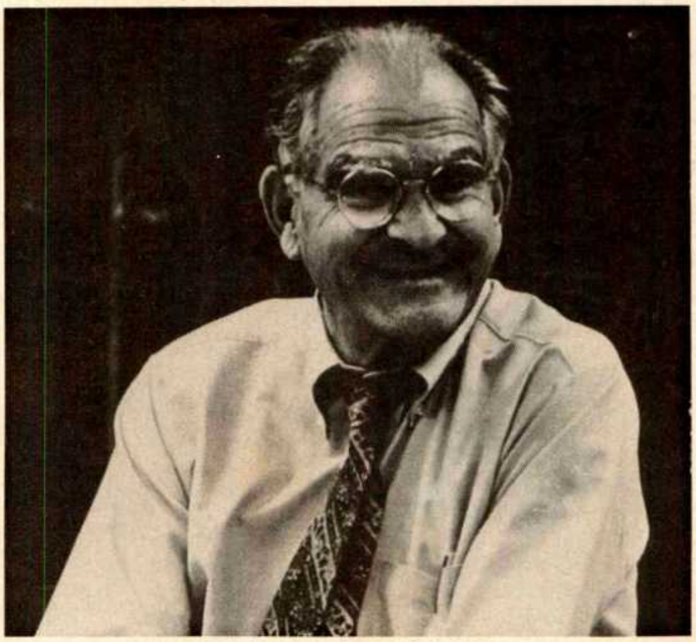
The Joy of Insight: Passions of a Physicist. By Victor Weisskopf. *Basic Books: 1991. Pp.336. $24.95.*

VICTOR Weisskopf, is a fortunate and happy ndividual, and he does not mind telling us so. Born in Vienna in 1908 to a well-to-do and intensely cultured Jewish family, Weisskopf lived through the Nazi destruction of European culture and science without harm o himself or any of his immediate family. An émigré in Rochester, then a Manhattan Project group leader at Los Alamos, and later an active citizen in the United States on issues of arms control and nuclear disarmament, he returned to Europe to play a significant role in the re-emergence of large-scale European science at CERN in the 1960s. Subsequently he has been a much-honoured senior statesman of science, a fixture of the Cambridge, Massachusetts, and world scenes, a man who summers in his '*petit paradis*', a chalet on the slope of the Jura Mountains with a view of Mont Blanc.

And what physicist would not trade 10,000 citations in the *Science Citation Index* for a chance to have been where Weisskopf was, so often at the very centre of the action of twentieth-century physics? As a student in gymnasium, he read voraciously the popular accounts of the then infant quantum mechanics, wrote a letter to Planck and received a postcard reply (a wrong explanation, we now know). Hans Thirring, his undergraduate mentor in Vienna, sent him on to Göttingen, where he studied under Max Born, James Franck, Paul Ehrenfest and Richard Courant, among others. For recreation there were trips to Berlin to see the musical theatre of Brecht and Weill and their followers, or a week in Jena listening to Furtwängler conducting Brahms.

In 1931, doctorate in hand, Weisskopf went to Leipzig, to work with Heisenberg (alongside Yoshio Nishina and Felix Bloch); Schrödinger invited him to Berlin as his assistant. "Often I saw the Nazi gangs . . . beat up Jewish students or those who looked Jewish . . . More than once I had to pull one of the boys under attack into my office so that he could escape through the back door." Weisskopf spent a year in Copenhagen with Niels Bohr (where he met his future wife, Ellen), then several years as Pauli's assistant in Zürich. Expelled by the Swiss *Fremdenpolizei* for alleged leftist connections (a bureaucratic fabrication, he says), Viki and Ellen spent some months in Copenhagen and Vienna, and crossed the Atlantic in 1937.

After the war and Los Alamos, Weisskopf was recruited to the Massachusetts Institute of Technology, where he became a powerful department head, wrote a classic textbook on theoretical nuclear physics, and supervised graduate students and postdoctoral fellows including Sid Drell, Murray Gell-Mann, David Jackson and Kurt Gottfried. Weisskopf was director-general of CERN from 1961 to 1965, during which time the decision to build a colliding-beam accelerator was taken.



Victor Weisskopf — a life fulfilled.

When we read the autobiographical story of a charmed life, we are not surprised to find it told by a charmer. Weisskopf is the very model of the modern, liberal, politically conscious and administratively adept, twentieth-century physicist, a major-domo of 'big science'. He did about everything there was to do, except make a major scientific discovery himself. Weisskopf's role has always been that of the facilitator, the brilliant and fair-minded administrator, the writer of textbooks and review articles and the inspiring lecturer capable of explaining the big picture. "It is regrettable," he writes, "that among scientists the presentation of ideas is not as highly valued as the creation of ideas. This is in stark contrast to music, where the performer is a partner equal to the composer."

If it did not ring so true, Weisskopf's memoir, *The Joy of Insight,* would seem intolerably smug. For example, "We were an unusual community, an international crowd of extremely creative people, and even our informal social gatherings were extraordinarily stimulating and interesting." (This could refer to any period in his life; it happens to be at Los Alamos.) When a rare cloud does shade this author's sunny disposition, it often has something to do with the question, what are sources of self-esteem for the almost-great scientist who has missed forever the young person's chance of making a truly important discovery? How many honours from professional societies and governments, how many good friends, splendid concert or theatre evenings, would one trade for a really important 'joy of insight' — important enough to make one's name immortal? Weisskopf recognizes that, before the war, he might have been able to make discoveries in quantum field theory that were in the event made by others after it. If only he had been a bit more mathematical. If only he had worked a bit harder, or loved music and socializing a bit less. But, on balance, he has few regrets.

In one of this book's rare sardonic passages, Weisskopf writes: "There are three periods in the life of a successful theoretical physicist. The first, when one is young, is the time of hard work on new ideas . . . In the second period one has become reasonably well known and is invited to give talks at various universities and at conferences. One still contributes valuable original work, but more often with the help of younger colleagues or by suggesting a line of research to them. In the third period one receives invitations to give general talks surveying the whole field through the light of experience. A little later one is invited to deliver memorial speeches about deceased colleagues or talks on the philosophical and political aspects of science. Finally, all one is asked to do is give after-dinner speeches."

Such shadows aside, Vienna, Göttingen, Copenhagen, Los Alamos, Cambridge and Geneva all make for a warm and engaging collection of stories, especially when they have been polished to a high sheen by years of retelling, and are now finally written down from the perspective of a mellow and much honoured old age. In a final chapter, the author discusses his personal, ranked choices of the greatest musical compositions and, especially, operas. ☐

William H. Press is in the Center of Astrophysics, Harvard University, 60 Garden Street, Cambridge, Massachusetts 02138, USA.

Molecular oncology

Antony J. Michalski

Genes and Cancer. Edited by Desmond Carney and Karol Sikora. *Wiley: 1990. Pp. 348. £24.95, $49.50.*
Molecular Genetics and Cancer Diagnosis. By Jeffrey Crossman. *Elsevier: 1990. Pp. 465. Dfl165, $69.50.*

The last two decades have seen an exponential rise in the amount and diversity of research in molecular biology. More recently, results of this technology have made an impact on clinical practice. Recombinant DNA techniques are used to produce growth hormone, insulin and haemopoietic growth factors. Identification of relevant restriction-fragment length polymorphisms is now routine in carrier detection and prenatal diagnosis of genetic diseases. The polymerase chain reaction is being used to amplify virus-specific sequences in the diagnosis of various virus infections, for example, human immunodeficiency virus in neonates. The genetic events responsible for tumorigenesis in retinoblastoma and colon cancer have now been identified and huge steps are being made in understanding the molecular pathogenesis of Wilms' tumour, Burkitts lymphoma and many other malignancies. No longer can clinicians and 'nonmolecular' scientists remain ignorant of the basic principles, techniques and jargon of molecular biology and they need a succinct and understandable introduction to this highly complex field. *Genes and Cancer* edited by Desmond Carney and Karol Sikora, and *Molecular Genetics and Cancer Diagnosis* by Jeffrey Crossman are aimed at a general readership and attempt to fulfil this role, though in different ways.

Genes and Cancer is divided into six sections covering oncogenes, gene control, tumour biology, growth factors and receptors, drug resistance and the use of antibodies for cancer therapy. The sections contain between four and eight articles of around ten pages in length and are followed by short but well-chosen lists of further reading. The authorship is largely UK based but there are contributions from the United States and Japan. The strength of the book is its broad scope and the many chapters that provide excellent reviews of a topic with the minimum of extraneous detail. In any evolving field, deciding on the balance between communicating hard data and speculative interpretation is difficult, but some chapters are made less digestible for a general readership by the inclusion of the full sequence of an oncogene or the configuration of its protein product. It is impossible for a book of this size to be comprehensive but I was surprised not to find a single chapter on 'tumour-suppressor' genes. Similarly, although paediatric malignancy numerically accounts for a tiny proportion of all cancer, it could have received more space if only to use retinoblastoma as a vehicle to discuss tumour-suppressor genes or Wilms' tumour to discuss genomic imprinting.

By contrast *Molecular Genetics in Cancer Diagnosis* is narrower in scope but more cohesive, with themes progressively developed by a variety of authors. It is divided into 25 chapters with up to 12 subheadings in each. The authorship is virtually all North American and has chosen to give comprehensive, but rather daunting, reading lists at the end of each chapter. The book concentrates much more on the application of formal molecular biology to oncology and does not attempt to cover current or future treatment modalities in any detail. The first eight chapters form a well-written, succinct introduction to the genetic basic of cancer, molecular techniques, oncogenes, tumour-suppressor genes, growth factors, metastasis and drug resistance. The chapter on the cytogenetic basis for molecular analysis of malignancies is particularly useful. The remaining chapters discuss the molecular pathology of selected malignancies. The majority are 'disease based' explaining the relevance of cytogenetic abnormalities, oncogene activation and loss of tumour-suppressor gene function to the tumour under discussion. There are several chapters on haematological malignancies but the solid tumours receive less complete coverage in terms of the variety of diagnoses considered, though the articles themselves are amongst the best in the book. Although some of the chapters are more detailed than others, and a few communicate data in a more philatelic than interpretative way, this is a readable and informative book. □

Antony J. Michalski is in the Department o Haematology and Oncology, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK.

Awaiting tests

Neil Turok

Particle Physics and Inflationary Cosmology. By Andrei D. Linde. *Harwood: 1990. Pp.362. £32, $60.*

The idea that one or more phase transitions occurred in the early Universe lies behind most of the theoretical work in cosmology over the last two decades. *Particle Physics and Inflationary Cosmology* is one of the first books devoted to a detailed technical treatment. Its author has been intimately involved in the subject since its inception, and has consistently been one of the most active and imaginative workers in the field. In the early 1970s, Andrei Linde was one of the first to realize that the standard electroweak theory predicts such a phase transition, and in the early 1980s played a leading role developing the inflationary model, through which the dynamics of a grand unified phase transition could resolve many of the basic conundrums of cosmology.

Linde has since then pursued new models of inflation, and their consequences, with great vigour. His particular strength is in finding simple physical arguments to clarify and explain the very complex processes involved. This comes through strongly in his book, which provides simple but quite deep treatments of many of the more technical issues — effective potentials, spontaneous symmetry breaking, the generation of fluctuations. It should serve as both a textbook and a standard reference on inflation for years to come. It is packed with original and intuitive viewpoints on finite-temperature field theory, inflation and quantum gravity. The emphasis is always on simple physical pictures and rough scaling arguments rather than on rigorous mathematical formalism. This can be a little frustrating — for example in the discussion of quantum gravitational effects for which there is as yet no consistent theory but which are treated in a crude intuitive manner. But Linde's point of view is always simple and stimulating.

The first half of the book is a nice review of phase transitions in field theories, including those in the standard model, bubble nucleation and topological defects. The main emphasis in the second half of the book is on an enthusiastic account of the many versions of inflation and in particular of two models: 'new inflation' and 'chaotic inflation'. The latter has come to be seen as probably the simplest embodiment of the inflationary hypothesis. But chaotic inflation is still on rather shaky footing: the initial conditions 'before' it are discussed using very rough arguments close to the Planck era, where unknown quantum gravity effects are undoubtedly important. This vagueness lends a note of unreality to the discussion. The book closes with a long heuristic discussion of quantum cosmology, the 'self-regenerating universe' which is a development of the chaotic model, and the anthropic principle. Somewhat disappointingly it thus ends firmly in the realm of philosophy rather than experiment.

My main criticism of the book is that it contains barely a mention of the observational data. Linde's primary concern is with the basic aesthetic problems of the hot Big Bang, and their resolution in theories of inflation, for which he is a very strong and able proponent. Inflation provides a simple dynamical mechanism through which a small lumpy 'typical' universe could become large flat and smooth like our own. At present there is no other mechanism known which does this, barring the supposition of special initial conditions, which may in the end always resolve these issues. Furthermore inflation provides a mechanism for the generation of small—amplitude primordial 'wrinkles' on the universe, which later grow under gravitational instability to form stars, galaxies and superclusters.

These successes have led some to speak of the 'inflationary paradigm', and to presume that the basic ideas of inflation are correct. In my view at least, that is a little premature. Experiment is surely an integral part of scientific progress, and our theories are worth very little if they are not testable. Of course there are fundamental limits to this in cosmology due simply to our inability to repeat or observe the early stages of the Big Bang. It is also conceivable that a theory might be so mathematically compelling that we might accept its validity even in situations far from those experimentally accessible. Such is the case, for example, in general relativity where most practitioners believe the theory correctly describes what would happen if one fell across the Schwarzchild horizon of a black hole. The various models of inflation are very far from this level of aestheticism, a fact their great number testifies to. This does not mean they are wrong — the standard model of particle physics, which is in wonderful agreement with a huge amount of real data, is actually pretty ugly. But models of inflation do require experimental verification.

The exciting thing about cosmology today, and what is really new about it as a science, is that this is no longer out of the question. The predictions of inflation for the form of the density fluctuations in the universe are eminently testable. First, by observations of the galaxy distribution on very large scales. Recent observations have actually shown significant disagreement with the inflationary predictions. Second, and probably more 'cleanly', inflation makes clear predictions for the precise form of the microwave–background anisotropy. Microwave–background experiments have reached a level of precision where they are close to ruling out or confirming most cosmological models for the large-scale structure of the universe, including those discussed in Linde's book.

Despite its neglect of these issues, I warmly recommend *Particle Physics and Inflationary Cosmology* both to serious students and researchers in the field. Linde's bubbly enthusiasm for a hundred theories and models at once provides both a stimulation for theorists and a challenge for experimentalists. ☐

Neil Turok is in the Department of Physics, Princeton University, Jadwin Hall, PO Box 708, Princeton, New Jersey 08544, USA.

New in paperback

- *Bivalve Filter Feeding: Hydrodynamics, Bioenergetics, Physiology and Ecology* by C. Barker Jorgensen focuses on filter-feeding bivalves and the ecological and physiological factors that determine the rate of filtration. Published by Olsen and Olsen at £22.
- From Oxford University Press comes *Quantum Description of High Resolution NMR in Liquids* by Maurice Goldman for those with no working knowledge of quantum calculations. Price is £17.50, $35.

Central player

Richard I. Gumport

Nucleic Acids in Chemistry and Biology. Edited by G. Michael Blackburn and Michael J. Gait. *Oxford University Press: 1990. Pp.446. Hbk £50, $115; Pbk £22.50, $49.95.*

A COROLLARY to the oft-stated theorem that 'the future is biology' is that 'the future of biology is chemistry'. For students wishing to begin acquiring the knowledge necessary to fulfil the corollary, *Nucleic Acids in Chemistry and Biology* is an excellent textbook. Although the text will be most useful to advanced undergraduate and beginning graduate students, practising chemists and molecular biologists who wish to garner a molecular understanding of the nucleic acids will also benefit from reading it. Students will require preparation in organic and physical chemistry and biochemistry to exploit the book fully, but little previous exposure to molecular biology is required. By stressing the pedagogical virtues of the text in this review, I do not mean to indicate that scientists actively engaged in research on nucleic acids will not learn by perusing the text, for it collects and presents information according to the judgements and perspectives of authors who are major contributors in their fields.

After a brief introduction in which the history of nucleic-acids research is interestingly recounted, the editors provide nine chapters written by themselves and selected specialists from Britain and the United States. The writing is clear and concise, and the editors have generally imposed a uniformity of style and depth of coverage upon the contributors that leads to overall coherence. Key words are in boldface and a relatively complete glossary with succinct definitions is provided. The major sections of each chapter have boxed, concise summaries that highlight the essential content. The many chemical structures and drawn-out reactions exemplify the emphasis of the text. Numerous well-conceived figures, some coloured in two tones for clarity and others depicted for stereoscopic viewing, contribute to the presentation. A comprehensive index further indicates the authors' intent to facilitate the use of the book by students. References to authoritative reviews and original research literature for each section of the chapters are provided. In many instances it is unfortunate that these are not designated in the text when the referenced topic is covered.

The body of the text begins with a consideration of DNA and RNA structure; the material proceeds from the heterocyclic bases through chromatin. The families of DNA duplexes, 'unusual' DNA structures, that is, cruciforms, curved DNA, supercoiling, and triple-strand nucleic-acid structures as well as a brief overview of the dynamics of nucleic-acid structures are covered. In the longest and most detailed chapter, the chemical syntheses of nucleosides, nucleotides and oligoribonucleotides and oligodeoxyribonucleotides are presented. The use of enzymes in synthesizing and joining oligonucleotides is also outlined. A short chapter that provides an overview of the biosynthesis of nucleotides and nucleic acids ends with a consideration of how drugs are used to inhibit some of these reactions. The following two chapters on DNA and RNA sequence information and transmission are presented at a very basic and introductory level. Gene structure, gene expression and its regulation, RNA synthesis and catalysis, and protein synthesis are outlined. The chapters will be most useful to those with chemical backgrounds who wish to begin to understand why there is such emphasis on the nucleic acids in modern biological research.

The remainder of the book is concerned with specific aspects of nucleic-acid chemistry. A relatively detailed chapter considers the chemistry of covalent interactions between small molecules and nucleic acids. A brief overview of the biological consequences of such modifications and of DNA repair concludes this topic. The chapter considering the noncovalent interactions of small molecules with nucleic acids covers groove-binders, intercalators, bisintercalators and DNA cleavage reagents. A chapter devoted to protein-DNA interactions starts with the forces that stabilize the complexes and proceeds through nonspecific to sequence-specific interactions. Structures of DNA-binding proteins, repressor-operator complexes, and an enzyme-DNA complex derived from X-ray diffraction studies are presented to explain the various mechanisms by which specificity arises. The contributions of using base modifications to study specific interactions are also presented. The final chapter is a basic introduction to genetic recombination with the emphasis on DNA and not the battery of enzymes and proteins that effect the processes. Homologous, site-specific, illegitimate and transposition recombination are presented. The chapter ends with an outline of cloning, recombinant DNA technology, *in vitro* mutagenesis, and a consideration of gene replacement.

There are many current textbooks available on molecular biology, but few that focus on the chemistry of a central player — the nucleic acids. This text fills that void with introductory, authoritative and up-to-date coverage. In spite of a few niggling errors that are inevitable in first printings and the occasional overstatements made during the necessary generalizations of an introductory work, this text will be extremely useful. Many students may be started along exciting and fruitful paths and some of their professors have their research itineraries modified as a result of studying this text. ☐

Richard I. Gumport is in the Department of Biochemistry, University of Illinois, Urbana, Illinois 61801, USA.

Falling victim to politics

Stuart L. Pimm

The Expendable Future: US Politics and the Protection of Biological Diversity. By Richard Tobin. *Duke University Press: 1991. Pp.325. Hbk £42.50, $45; pbk £17, $18.75*

As species extinction accelerates worldwide, we look in despair at developing, tropical, countries that sacrifice biological riches for short-term economic gains, or even poorer reasons. Surely, in a country like the United States, with an affluent and well-educated population, circumstances should be different. As Richard Tobin explains at the beginning of *The Expendable Future*, the great majority of Americans believe that we should prevent extinctions. He quickly notices that surveys indicate that about 60 per cent of people with these views, also feel that "modifying the environment for human use seldom causes serious problems", and that our species "was created to rule the rest of nature". Thus, there is likely to be a wide gap between expectations and reality. Tobin documents that gap with clear writing and carefully constructed expectations of what should not, but inevitable do, fall victim to politics.

The first expectation, given the majority view, is that the US agency mainly responsible for protecting diversity, the Fish and Wildlife Service (FWS), should be well-placed within the administrative structure. In fact, it is buried within a bureaucracy, surrounded by agencies largely devoted to the subjugation of nature. Whatever its location, the FWS can assert itself in several ways. The most crucial stage in the protection of endangered species is the first — the listing of species on the brink of extinction. This listing process began in the early 1970s with high expectations. In 1975, with the help of the Smithsonian Institution, over 3,000 species of plants were suggested as possible candidates. The Endangered Species Act, however, contained a short, apparently innocuous paragraph that required all federally-funded projects to not jeopardize endangered species. FWS listed the snail darter, (a small fish), challenged the Tennessee Valley Authority's construction of the already highly controversial Tellico Dam, and won in the US Supreme Court. The local senator, Howard Baker, had to introduce special legislation to permit the dam to be finished. Listing species became a difficult and very slow process.

The FWS maintains that its decisions to list species are based on "the best scientific and commercial data available" — just as the law requires. Listing should be on the basis of threat, not on political expediency, or whether the animal or plant is small and slimy or cuddly and large. Globally endangered species (particularly if in a monotypic genus), should receive priority over locally endangered populations of an otherwise flourishing species. Listing should be more frequent in Hawaii where most of the endangered species are to be found, than in the continental United States. In practice, the species listed tend to be local populations of large birds or mammals, in mainland areas where they cause few impediments.

In addition to listing , the FWS can designate critical habitats for species. Again expectations were high, and again, the resulting controversy often considerable, and the subsequent actions few. Rather more tangible is the acquisition of land to house endangered species. Tobin states that "considerable amounts of money have been devoted to lease purchase habitats for subspecies facing a low or medium threat and having doubtful prospects for recovery." Bald eagles have done very well financially, despite a FWS assessment that the species is in no immediate danger. Finally, there are the recovery plans — the actions proscribed to return species to better times. Again, successes are few.

The author is a political scientist and presents the organizational history, political decisions, and changing laws and administrative structures in ways that as an ecologist I found compelling and interesting. He concludes with recommendations for the politicians, lawyers and administrators. But what should we ecologists get from this book? First, the book infuses a sense of political reality: the federal government will not save small ugly invertebrates with funny sounding names; we should seek help for these species elsewhere. Second, in the decisions to save species and the habitats that house them, ecologists could do much more to become informed about where endangered species are to be found. Spending over $20 million to save the Mississippi sandhill crane (a subspecies) and more on bald eagles than on any other single species, while very much less to save the very large number of critically endangered species of plants in the tropical forests of US states and territories (Hawaii, Guam and other Pacific areas, Puerto Rico and other Caribbean areas) is inexcusably provincial. How can we save tropical forests worldwide if we cannot save them at home? Ecologists need to know where the diversity is, where it is threatened, and be more assertive in their priorities. Finally, there is considerable scientific uncertainty about how to determine the relative threats to species and the likelihood of recovery. Without better ecology, endangered species are going to remain potential victims of political whims. □

Symbol of America — the bald eagle is in no immediate danger yet receives more financial assistance than any other species. Picture from *Hawks, Eagles and Falcons of North America* by Paul A. Johnsgard (Smithsonian Institution Press, $45, £29.95).

Stuart L. Pimm is at the Department of Zoology and Graduate Program in Ecology, The University of Tennessee, Knoxville, Tennessee, 37996, USA.

Food web patterns and their consequences

Stuart L. Pimm, John H. Lawton & Joel E. Cohen

A food web is a map that describes which kinds of organisms in a community eat which other kinds. A web helps picture how a community is put together and how it works. Although webs were often initially reported in despair at ever understanding ecological complexity, recently discovered widespread patterns in the shapes of webs, and theoretical explanations for these patterns, indicate that webs are orderly and intelligible, and have some foreseeable consequences for the dynamics of communities.

FOOD webs are the road-maps through Darwin's famous 'entangled bank'[1] and have a long history in ecology[2]. Like maps of unfamiliar ground, food webs appear bewilderingly complex. They were often published to make just that point. Yet recent studies have shown that food webs from a wide range of terrestrial, freshwater, and marine communities share a remarkable list of patterns. Current research concentrates on how many independent patterns there are, how they may be described quantitatively, why the patterns are so general, and what are the consequences of these patterns for the dynamics of a community and its constituent species. Just as any map omits details, most published webs omit predation on minor species, the quantities of food consumed, the chemical composition and temporal variation of the flows and many other details. Published webs are also of very variable quality. These omissions and problems are causes of concern, but on present evidence do not present insurmountable difficulties.

The data

Food web complexity is exemplified in Fig. 1. (This web[3] predates Shelford's study[4], which is often credited as the first published web, by one year.) The community embracing the boll weevil is certainly complex, but not hopelessly so; indeed it raises several issues of contemporary interest. For instance, what level of taxonomic resolution is appropriate for work of this kind (most taxa in Fig. 1 are highly aggregated, but some are identified to species); and how are the boundaries of the studied system to be delineated?

Three kinds of food webs are published. A source web includes one or more kinds of organisms, the organisms that eat them, their predators and so on (for example, the part of Fig. 1 based on the cotton plant). A sink web describes one or more kinds of organisms, the organisms they eat, plus their prey, and so on. A community web is defined by picking, within a habitat or set of habitats, a group of species without regard to the eating relations between them, and working out who eats whom[5]. Reference 6 compiles 113 community webs and most of these webs contain between 5 and 50 species. We focus here on community webs[5-7], an example of which is shown in Fig. 2. Terms used to describe web features and the information that can be extracted from them are defined in Boxes 1 and 2. Over 200 community webs have been reported in the literature[7] (available in machine-readable form through ECOWeB[7]) embracing a wide variety of habitats, locations and taxa. The analyses of these webs are usually in terms of trophic species—a set of organisms with identical prey species and identical predators, within the level of resolution used in the study. Figure 1 is unusual in that it indicates the number of species grouped into a trophic species; there are, for example, 12 species grouped under the trophic species of 'leaf worm parasite'. In general, a trophic species may correspond to a set of biological species, to a single biological species or to a single life-stage of a species. We shall use 'species' to mean 'trophic species' throughout.

Features common to all webs

Box 1 explains the statistics commonly derived from a web and also gives the particular statistics derived from the *Nepenthes* web. Two associated graphs, the predator overlap graph (also called the trophic niche overlap graph[5] or, by graph theorists, the competition graph) and the prey overlap graph[8] are derived from an analysis of the web (Box 2 and Figs 3 and 4). Several features are common to the set of webs studied so far, a set that has widely varying numbers of species and contains webs from many different habitats. Common features (see Box 3) include the average proportions of top, intermediate and basal species, the ratio of predatory species to species of prey, the proportions of trophic linkages between different kinds of species, the length of food chains, the absence of compartments within a habitat, and complex patterns in the topological relationships between predators and prey elucidated by the predator and prey overlap graphs.

Are the patterns real?

Are these general patterns artefacts? There are good reasons for concern about the quality of data in published webs. Communities often contain thousands of species. Because published

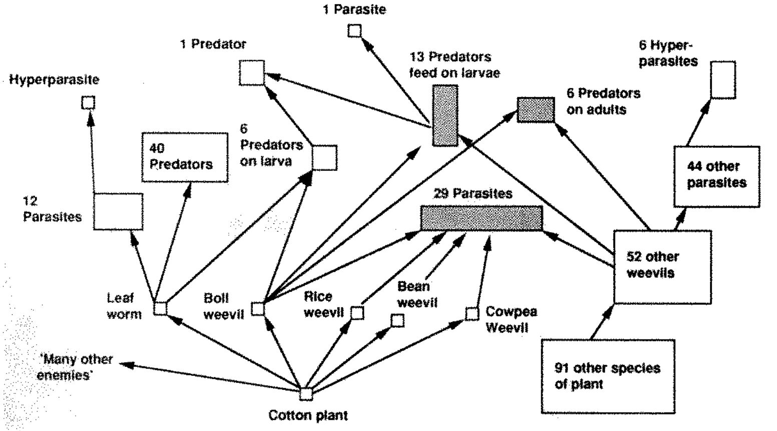


FIG. 1 The food web associated with the cotton plant and the boll weevil (after ref. 3). The cotton is attacked by 'many other enemies' than those specified. Moreover, the predators shown in the three shaded boxes are known to attack species other than those shown in the figure.

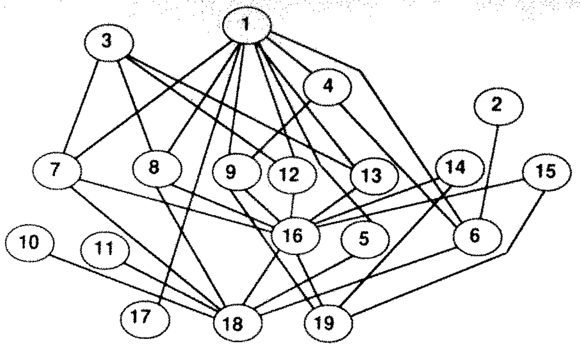


FIG. 2 A food web of the insects in the pitcher plant *Nepenthes albomarginata* in West Malaysia (from ref. 16). Each line represents a trophic linkage; predators are higher in the figure than their prey. Key: (1) *Misumenops nepenthicola*; (2) Encyrtid (near *Trachinaephagus*); (3) *Toxorhynchites klossi*; (4) *Lestodiplosis syringopais*; (5) *Megaselia* sp. (?*nepenthina*); (6) *Endonepenthia schuitemakeri*; (7) *Triperoides tenax*; (8) *T. bambusa*; (9) *Dasyhelea nepenthicola*; (10) *Nepenthosyrphus* sp.; (11) *Pierretia urceola*; (12) *Culex curtipalpis*; (13) *C. lucaris*; (14) *Anotidae* sp. 1; (15) *Anotidae* sp. 2; (16) bacteria and protozoa; (17) live insects; (18) recently drowned insects; (19) older organic debris.

webs include only tens of trophic species (>100 is exceptional), they are either highly aggregated or represent only a tiny part of the entire system. Aggregation is rife in many published webs; moreover, aggegation varies in extent from web to web, and at different positions in the same web[9]. Even when webs are detailed enough for most of their elements to be single biological species (for example Fig. 2), the linkages are less often based on experimental evidence than on casual observations; only those linkages that are 'artistically convenient'[10] may appear in the figure. In recent studies we detect a counter-tendency to report every linkage, no matter how minor. We appeal for studies where the frequencies of feeding encounters are reported, so that objective criteria may be used to include or exclude linkages.

There are a number of rejoinders to the criticisms that the data are too aggregated and unreliable to reveal meaningful patterns. Possible artefacts ought not to apply equally to webs from special microhabitats described in detail and to those more aggregated webs summarizing interactions throughout larger communities. Present evidence suggests that most patterns appear across all webs. Moreover, artefacts should be more obvious in the highly aggregated webs. Indeed, the ratio of species of predators to species of prey is sensitive to the greater aggregation of species at lower trophic levels[9], and the ratio itself has dropped as better data have become available[11,12]. Nevertheless, the average proportions of species at different levels change only slowly or not at all across webs differing widely in numbers of species and degree of aggregation, though the variation in these proportions is large. Detailed webs can be progressively aggregated. For instance in Fig. 2, the three pairs of biological species (10 and 11; 12 and 13; 14 and 15) that have the same sets of predators and prey could be joined into three trophic species. The web could be further aggregated by joining 7, 8, 12 and 13, which share similar species of predators and prey. Aggregation of webs using trophic criteria affects webs' properties only slightly[13]; in contrast, progressive aggregation by taxonomic affinities alters webs' properties more rapidly (K. Schoenly and G. Sugihara, manuscript in preparation).

Recent studies to address limitations in the data (for example, refs 14–18) have generally confirmed the patterns. The pattern

BOX 1. Definitions of web features and analysis of the *Nepenthes* web

Top predators are species on which nothing else in the web feeds, basal species feed on nothing within the web, and intermediate species have both predators and prey within the web. Linkages are trophic connections between species.

Cycles occur where, for example **j** eats **i** and **i** eats **j**, or **i** eats **j**, **j** eats **k** and **k** eats **i**. Cannibalism is a cycle where one species feeds upon itself.

Connectance is the number of realized trophic interactions divided by the number of possible interactions. Within this definition are several closely related variants. The number of possible interactions may be S^2 if cannibalistic interactions are counted, $S(S-1)$ if only interspecific interactions are counted. We may assume that for each linkage between predator and prey there are two interactions: the effect of the predator on the prey, and the effect of the prey on the predator. Alternatively, we may assume that either of these two effects is dynamically zero. Typically, connectance is calculated from twice the number of observed linkages divided by $S(S-1)$ for the number of possible interactions. Hence, when the number of links per species (linkage density, d), is constant, connectance declines approximately hyperbolically with increasing number of species.

The trophic level of a species in webs of linear structure is one more than the chain length, that is, the number of linkages between it and the basal species in the web (which may be plants or detritus). In reality, species may trace linkages to basal species along food chain pathways of different lengths.

Omnivores are species that feed on more than one trophic level[9]. Omnivores blur the distinction between trophic levels, because top predators may trace linkages to basal species through food chains of different lengths. Omnivory can involve either different food chains or the same food chain. In the latter case, for example, an omnivore may feed on both a prey species and that prey species' prey species. Other definitions of omnivory are possible[20].

Compartments exist when linkages are few (or weak) between groups of species and common (or strong) within those groups.

Species analysis
Number of top predators is 7: species **1**, **2**, **3**, **10**, **11**, **14**, **15**.
Number of basal species is 3: species **17**, **18**, **19**.
Number of intermediate species is 9: the rest.
Number of linkages: intermediate to top 14; basal to top 4; intermediate to intermediate 8; basal to intermediate 7.

Cycles. There are no cycles in this web.

Connectance and linkage density.
Connectance = 0.19 (calculated as twice the number of linkages divided by $S(S-1)$. Linkage density = 33/19 = 1.74.

Chain lengths
Minimum 1: for example, species **1** to **17**, **15** to **19**. Maximum 4: species **1** to **4** to **9** to **16** to **19**.
Modal number for each top predator: species (Mode)
1 (3); **2** (2); **3** (3); **10** (1); **11** (1); **14** (2); **15** (2).

Omnivory. In this web omnivory occurs via different chains, for example, species **1** to **17** and **1** to **18** and **19** via longer pathways, and also within chains, for example, species **1** to **5** and **1** to **4** to **5** or species **9** to **19** and **9** to **16** to **19**.

Compartments
None in this web.

There may be a correlation between the number of species of prey each intermediate species exploits and the number of species of predators that exploit the intermediate species

Intermediate species	**4**	**5**	**6**	**7**	**8**	**9**	**12**	**13**	**16**
No. of prey	2	1	1	2	2	2	1	1	2
No. of predators	1	1	3	2	2	2	2	2	7

least likely to survive detailed scrutiny involves the linkage density, d, which is the number of trophic linkages L per (trophic) species S. Averaged over webs in the range from 3 to 48 species, the average number of linkages ($E(L)$) is roughly twice the number of species[6] in any given web (that is, $E(L) = 2S$; $d = 2$). This is equivalent to a hyperbolic relation between connectivity C and species richness S (see linkage density in Box 3). In the original description of this pattern[6] it was noted that a power-law $E(L) = kS^{1+\varepsilon}$, for some small positive ε, was also a viable description of the data, and that future data on webs with large numbers of species would have to distinguish the alternatives. With a few larger webs in hand, a power law with ε probably between 0.3 and 0.4 indeed seems reasonable[6,19].

What the patterns do not show

Although most analyses ignore the problem, web structure varies over time and space[15,20]. A useful distinction[21] is between cumulative webs gathered over many occasions (the majority of the published webs) and time-specific webs. In most of 16 habitats with reported time-specific webs, the percentages of top, intermediate and basal species fluctuated widely, generating appreciable variation in predator-to-prey ratios, mean chain length, and linkage density[21]. Within any one habitat, cumulative webs usually overestimate linkage density and underestimate the percentage of basal species relative to time-specific snapshots[21]. Web statistics are sensitive to occasional rare species (typically large, generalized predators) encountered on only some sampling occasions.

Comparable systems involving related taxa and/or similar habitats reveal differences in web statistics from place to place within one region[15] and locally within a single habitat[14]. The significance of these differences is not well understood.

The question of how to define the boundaries of a web is particularly vexing. Most investigators tacitly admit that members of most webs, particularly those higher in the food chain, feed outside the system studied. Published webs are reticulate, with no evidence of compartments (Box 3) except, possibly, fuzzy ones at habitat boundaries[9]. Given the apparently arbitrary nature of the boundaries of most studies, one conclusion must be that web statistics are roughly independent of spatial scale. This leaves open the question of whether spatial and temporal variation in web structure are interchangeable[21]. In certain physical systems, a single system observed over a long time period reveals variation that is identical to that observed in many systems of the same kind simultaneously but at different places. Interchangeability of temporal and spatial variability is called ergodicity; we do not know whether web statistics are ergodic.

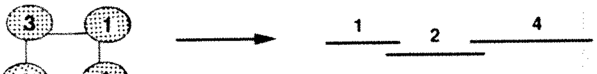
Differences between systems

Although webs have common features across a wide range of habitats and taxa, there is some variation. Counter to earlier predictions, average food-chain lengths do not seem to differ greatly among ecosystems with very different primary productivities[6,9]. There are only slight differences in chain lengths between communities where the consumers are often vertebrates compared with those where the consumers are all invertebrates[22,23]. Chains may be shorter in small habitat patches. Thus, on very small islands predators present on larger islands may be absent, and small ponds may lack the predators of larger lakes[19]. In some special microhabitats, chains are shorter in areas with frequent natural or experimental disturbances[24]. Reported chains are shorter in two-dimensional habitats such as grasslands and the intertidal than in three-dimensional habitats such as forests or the water column of lakes or oceans[6]. Intertidal ecologists, however, do not record the predatory fish that visit their systems at high tide, whereas those working below the low tide mark do. Hence, different average chain lengths may be an artefact of different academic traditions or, as discussed in ref. 19, a genuine difference in the effects fish have on bottom-living versus free-swimming prey. We caution against hasty and uncritical comparisons of web features, especially now that the availability of a collection in machine readable format[7] makes such comparisons easy. More convincing constrasts in patterns will be revealed within sets of webs specially collected for the purpose by the same scientists[11,14-18,20].

BOX 2. Predator and prey overlap graphs

From a given web, we can obtain another graph, called the predator overlap graph. In this graph, an edge connects predatory species that share one or more species of prey in their diets. There may be more than one set of connected predators: these sets are called components.

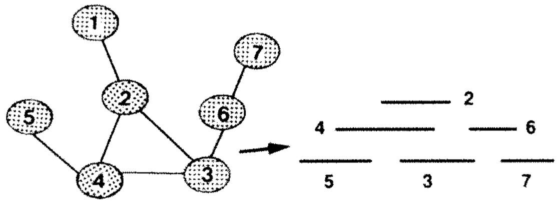
Predator overlap graphs are rigid circuit, when every circuit around four or more predators is divided into triangles by one or more edges across the circuit. Below is a hypothetical overlap graph that is not rigid circuit, it would be if an edge joined predators **1** and **4**, **2** and **3**, or both.



(3 cannot be placed)

A web is defined to be interval if each predator can be represented by a line segment and the overlaps in the predators' diets are exactly represented by the overlaps among the line segments. The hypothetical graph above left is not interval because the interval that represents predator **3** cannot overlap the intervals that represent predators **1** and **4** without also overlapping the interval that represents predator **2**, contrary to the graph.

For many arrangements of predator overlap graphs, the rigid circuit property ensures that the overlaps will have an interval representation. Yet the rigid circuit property does not guarantee that food webs are interval. Below is a non-interval, rigid-circuit predator overlap graph. There are no circuits around four or more species. It is still not possible to express the overlaps as overlapping segments of a line, because any segment for predator **1** that overlaps the segment for predator **2** must also overlap the segment for predator **3** or **4**, contrary to the graph. This pattern is called asteroidal and the example comes from 7 species of gastropods in the genus *Conus*, from ref. 29 and original work by ref. 55.



(1 cannot be placed)

Prey overlap graphs are formed by connecting prey species sharing one or more predators. Two prey sharing predators form a line, three a triangular plane, four a tetrahedron, and so on. Some prey species will be used by more than one predator and this allows us to assemble the individual graphs into a complete picture describing the relationships between predators and prey. Now consider a physical analogy of this overlap graph with the objects formed by connecting the various prey species that share a particular predator being considered to be solid. It is possible for this physical analogy to have holes; topological holes are their equivalent in the graph. Topological holes are rare in webs with small numbers of species[8], but Fig. 4 provides an example.

In web studies, omnivory has a special meaning (Box 1). Webs are said to contain omnivores when species can trace the energy they obtain through pathways of different lengths. Although still poorly studied critically by a single investigator, the extent of omnivory apparently varies between systems[9,19]. Omnivory is statistically rare in some webs, but there are at least three important exceptions. Aquatic webs often have as their top predators fishes that start their lives as very small zooplanktivores and thus 'eat their way up the food chain' as they grow. Detritivores often pay little attention to the former trophic position of species now dead. Finally, webs composed of insects and their parasitoids often contain species that feed facultatively on many different species at different trophic levels.

What causes the patterns?

How many independent web patterns are there? Which patterns are the consequences of others? What causes the patterns? A variety of evolutionary and natural history explanations have been put forward for particular web properties (reviewed in refs 9 and 25). Although we do not dismiss these explanations (most have been poorly investigated), we wish to focus on two more general explanations: the cascade model and web dynamics.

The cascade model

The cascade model[6] focuses on the static patterns of trophic interaction and assigns linkages at random subject to two constraints. First, the model assumes that species can be arranged *a priori* into a cascade or hierarchy such that a given species can feed only on species below it, and itself can be fed on only by species above it in the hierarchy. This ordering automatically precludes cycles (Fig. 2; Boxes 1 and 3) and decomposer loops. It does not specify whether any particular species must be top, intermediate, or basal (except the lowest and highest species in the cascade). Second, the model requires two parameters obtained empirically: S and d. By assumption, connectance declines hyperbolically.

By assigning linkages randomly within these constraints, the cascade model generates quantitative predictions that can be compared rigorously with observed patterns. It correctly predicts the average and variance of the fractions of all species that are basal, intermediate, and top predators (Box 3); the average fractions of linkages that are basal-intermediate, basal-top, intermediate-intermediate and intermediate-top (Box 3); the modal length of chains from basal to top species (Box 3); and the decline in the frequencies of interval and rigid-circuit predator overlap graphs (Box 3) as webs get larger.

The cascade model has not yet been used to explore some features of webs that merit attention, such as omnivory, compartments (Box 3), and the ratios of how many prey species a species exploits to how many predatory species that species suffers (Box 3). It also gets some of the fine details wrong though, again, problems in the quality of the data may be partly responsible for the discrepancy. For example, predicted declines in interval and rigid-circuit predator overlap graphs as S increases are too rapid, and the predicted frequencies of very short and very long food chains within a given web are too high.

The assumption of constant linkage density is challenged by data on species-rich webs (above). An increase in linkage density with the number of species has been incorporated in a variant of the cascade model[12] which, surprisingly, produces a poorer fit to some other observed patterns than the original model. Obviously, the cascade model is very simple, making it easy to refine in various ways, while retaining the essential feature of a trophic cascade. Of 13 such variants[12] only one does as well as or, in some cases, better than the original model in predicting observed web properties. This variant has yet to be tested in detail.

The original formulation of the model offered no explanation for the postulated trophic cascade. Body size is a likely candidate[26] because typically predators are larger[27] than their prey, and parasites are smaller[25,26]. The relationship between the size of a predator, the size of its prey, and their positions in the web is important[28] and not fully explored.

What determines the linkage density? Quantitative theory to explain why the average species apparently utilizes, and is utilized by, a predictable and fairly small number of other species is crucial to a deeper understanding and ultimately testing of the model. We offer one explanation below.

BOX 3. Features common to published community webs and their associated graphs

Features of the web itself

- Cycles are rare[5]. (The definitions of cycles and of other terms in this box are in Boxes 1 and 2.)
- The average proportion of top predators, intermediate species, and basal species remains roughly constant (but with high variance)[5] in webs with widely differing numbers of species and from different habitats.
- The average proportion of trophic links that are between intermediate and intermediate species, intermediate species and top predators, basal species and intermediate species, and basal species and top predators remains constant[5] (with large variance) in webs with widely differing numbers of species and from different habitats.
- Linkage density seems to be approximately constant for webs with few species, but may increase with large numbers of species (see text).
- For top predators, the modal food chain lengths are typically 2 or 3, with lengths of only 1 being less common (and perhaps representing incomplete information) and those greater than 3 being uncommon[5,9]. Correspondingly, the modal number of trophic levels is 3 or 4.
- Omnivory is rare in some webs, though there are many exceptions (see text).
- Habitat boundaries sometimes impose compartments on food webs. Compartments are not usually observed within habitats[9].
- For intermediate species, how do the numbers of species they exploit correlate with the numbers of species exploiting them? Studies of species at the same trophic levels in different systems (for instance insect herbivores or parasites) typically do not find a significant correlation[9,54]. Within a food web, species at higher trophic levels tend to have more prey and fewer predators than species at low trophic levels, an observation predicted by the cascade model[5].

Features of the predator overlap graphs

- Webs with small numbers of species only rarely have predator overlap graphs that are not rigid circuit[5].
- Food webs with small numbers of species usually have interval overlaps in the predators' use of prey species[5].
- For many arrangements of predator overlap graphs, the rigid circuit property ensures that the overlaps will be interval. Yet the rigid circuit property does not guarantee that food webs are interval because of the asteroidal pattern. Such asteroidal patterns of overlap are unusual in webs with small numbers of species[5].

Features of the prey overlap graphs

- Topological holes are rare in webs with small numbers of species[8].

Web dynamics

Most webs are static descriptions, but the communities they describe are not static. Some species are successful at invading a community whereas others are not and the successes may or may not cause extinctions of former residents. This process of assembly and disintegration may explain many of the empirical web patterns[9,29]. It is not incompatible with the cascade model because it suggests general mechanisms that limit linkage density and species richness, while making specific predictions about the details of web patterns.

The idea that web patterns are shaped by dynamic constraints on assembly and disintegration was first formulated using Lotka-Volterra equations[9,25]. (These are sets of quadratically nonlinear first-order constant coefficient differential equations. Each equation determines the rate of change of a species in the web, from the density of every species with which it interacts, as well as its intrinsic birth and death rates.) Such analyses distinguished stable (persistent) from unstable web structures and so concentrated on the disintegration of webs. In general, these models predict that systems with high linkage density d will be unstable; the critical value of d declines with increasing S. Recent models assembled complex communities from simpler ones and found comparable results: models with low d for a given S are more likely to be invaded[30-32]. Moreover, once d

and S have come into dynamic equilibrium, each successful invasion makes subsequent invasions less likely[32,33].

Although based on extremely simple models, the processes uncovered are often intuitively sensible, as can be seen from their specific predictions. Consider the difficulties of entering highly connected webs. If invading predators overlap in their prey use with existing predators, levels of interspecific competition will increase, reducing the chance of successful invasion or making the persistence of competitors already in the web less likely. Either way, this process limits species richness and linkage density. Less familiar is the process of apparent competition[34] between species of prey that share the same predators. An increase in one prey species may cause an increase in the shared predator and a consequent decrease in the other prey species. High linkage density makes shared predators more likely, apparent competition stronger and webs harder to invade as a consequence. Competition and apparent competition acting in concert may generate constant predator-to-prey ratios in webs[35].

Species may enter the top of existing food chains. Long chains, however, reduce the rate at which population densities recover from environmental disasters[9] making the persistence of species in long chains unlikely and invasion more difficult. This argument explains why chains appear shorter in unpredictable systems and conceivably explains why insect-dominated chains are short[22]. Insects have particularly variable population densities and may therefore have much larger minimum viable population densities than vertebrates[29].

The scarcity of omnivory in some systems may be due to the obvious difficulties encountered by a species that is both the prey and the competitor of a resident species. Alternatively, a successfully invading omnivore may locally exterminate a resident species on which it can both feed and with which it competes, so forming a food chain without omnivory. In contrast, when each of the omnivore's life history stages depends critically on resident species at progressively higher trophic levels as the omnivore matures, the omnivore cannot eliminate these intermediate species and still persist[36]. Dynamic models also predict that omnivory will be common in decomposer systems (because the consumers are donor-controlled[25]) and in communities of insects and their parasitoids[25]. Other approaches to dynamic modelling of food webs are under investigation[28].

Concatenating dynamic and cascade models

Dynamic models contrast in purpose, technique, and accomplishment with the cascade model. The dynamic models attempt to explain why some web features are rarely or frequently observed in nature, without saying exactly how rarely or how frequently. These models rest on several largely untested assumptions[37] but offer insight into large questions of community structure, stability and change that are currently beyond the scope of the cascade model. By contrast, the cascade model aims for quantitative explanation, unification and prediction of observed patterns. It rests on extremely simple assumptions that are susceptible to direct test (possibly after additional interpretation, as when the cascade is supposed to be an ordering by body size).

Careful observations and experiments on spatial and temporal variation in webs may reveal more about the roles of the cascade and the dynamic models. For example, seasonal changes in web structure may differ from successional changes. Dynamic models predict that unstable webs from early successional habitats should depart significantly from the norm, for example, in having unusual and widely fluctuating predator-to-prey ratios[35]. The predictions of the cascade model for this situation have yet to be developed.

In real ecological communities both population dynamics and trophic structure are important. A new hybrid model, the Lotka–Volterra cascade model (LVCM)[37], assumes the population dynamics of the Lotka–Volterra model when the interactions between species are shaped by a refinement of the cascade model. The LVCM displays an ecological phase transition: gradual changes in the probabilities of various kinds of interactions related to feeding produce rapid changes in a community's probability of qualitative stability. The frontier of stability in this model differs from that of a classical model of May[38]. The LVCM makes possible a unified discussion of stability and trophic structure in a single, analytically tractable framework, but inherits the shortcomings shared by its parents, the Lotka–Volterra equations and the cascade model.

Consequences of web patterns

The shape of food webs has inevitable dynamic consequences for constituent species. Our understanding of these consequences is growing quickly[29,39]. For example, the rate at which populations recover from disasters (resilience) theoretically should depend on food chain length[9], an idea now supported by a limited number of experimental studies[40]. Variability of population density depends on features of the species examined and on the variability of the physical environment. It also depends on web features such as the number of prey the species exploits, and the number of predatory species that exploit it[41,42].

Removal of individuals is a common experimental procedure in ecology[43-45]. Food web theory warns that it may be difficult to predict responses of unmanipulated species. For instance, permanent removal of a species' predator may ultimately cause the species' density to decline if the predator also fed on a powerful competitor. Theory predicts that even when the web is known, slight changes in the parameters that describe interspecific interactions may be amplified or dampened by complex pathways generating indirect effects between any pair of species[46-48]. In contrast to these final consequences of removals, there should be little doubt about the direction of the transient changes[46]. Thus, prey species should initially increase when their predators are removed: it takes time for indirect effects to appear. When we do see changes in density expected from immediate interactions with prey and predators, it may only be because of the typically short duration of field studies. At intermediate time scales, we should see effects rippling through the web, a feature illustrated by the relatively long-term studies of the removals of ants and rodents from desert communities[49].

Web theory has addressed changes in species composition, and in the total density of a group of species when species are manipulated or removed[29]. Theory predicts that highly-connected (complex) communities should be most sensitive to the loss of species from the top of the web because secondary extinctions propagate more widely than in loosely connected (simple) communities[29]. In contrast, simple communities should be more sensitive to the loss of plant species than complex

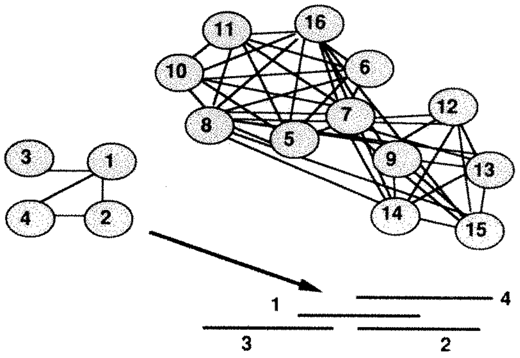


FIG. 3 Predator overlap graph for the *Nepenthes* web. In the *Nepenthes* web there are two connected sets of predators one involving four predators, the other involving the other twelve. The overlap graph in the component of the *Nepenthes* graph involving predators **1** through **4** is rigid circuit. Species **3** in the figure does not violate this condition because it is not part of a circuit around four or more species. In the *Nepenthes* web, the observed predator overlap involving species **1** to **4** is interval—as can be seen from the overlaps of the segments above right. (See Box 2 for terminology).

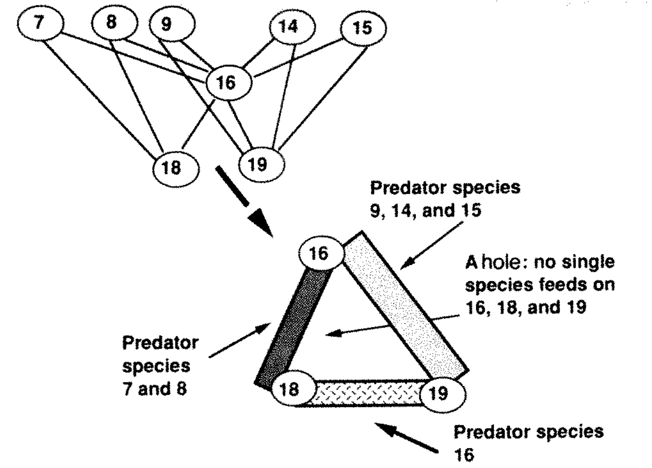

7
8
9
14
15
16
18
19
Predator species 9, 14, and 15
A hole: no single species feeds on 16, 18, and 19
16
Predator species 7 and 8
18
19
Predator species 16

FIG. 4 Prey overlap graph derived from the *Nepenthes* web and the 'topological hole' formed by connecting the various prey species that share a particular predator (see Box 2).

communities, because in simple communities the consumers are dependent on only a few species and cannot survive their loss[29]. 'Keystone' species, those having disproportionate effects when removed, can occur at all trophic levels. Theory[50] has also addressed empirical findings[51] that when grazers are removed from a community, increased plant diversity is associated with large changes in species composition, but relatively small changes in total plant density.

Whither food webs?

A major obstacle to progress in food web research is the weakness of the available data; this is more severe than a matter of not having enough webs. A bigger problem is the lack of methodological standards; the aim must be to reduce inter-observer subjective differences in the way in which field workers observe and report webs. To assess the completeness of observations, Cohen[5] recommended a yield-effort curve, which plots the cumulative number of species and linkages against sampling effort. In a recent example[52], there is little sign of a decrease in the number of new species observed per field day after more than 5,000 hours of observation over 10 years. This finding supports the criticism, often made, that many published webs are grossly incomplete in the species and perforce in the linkages included. In such detailed studies, however, many of the linkages must represent rare and thus possibly dynamically unimportant events. The need to quantify the relative frequency of feeding interactions is crucial.

Despite methodological and theoretical problems, we favour a cautiously optimistic view of the future of food web research. Present shortcomings in theory and data must serve as a spur to refining both; they are not a reason to discard emerging insights and understanding.

Nature presents ecologists with ecological communities as the natural units of analysis. Though boundaries may be drawn in different ways for different purposes, the given primitives of ecology are forests, lakes, watersheds, wetlands, shorelines, deserts, estuaries, oceans, and the like. Populations of a single species, and still more individuals of a single population, are abstractions from communities for the sake of analytical convenience. To a considerable degree, these abstractions have upstaged the communities from which ecologists isolate them. In this time of rapid environmental change, the many endangered species are only a drop in the flood of endangered communities. Although it is by no means the only, or a sufficient approach, a quantitative, predictive understanding of food webs would provide a better basis for solving many problems of applied ecology than is now available. The average proportions of species in different trophic positions is found to be roughly invariant in webs with different numbers of species: this finding has already been used, for example, in an estimate of the total number of species on earth from the number of known plant species[53]. We anticipate increasing use of food web theory in such disparate but pressing problems as the management of multispecies fisheries, integrated pest control, and predicting the effects of climate change on ecological communities. ☐

Stuart L. Pimm is at the Department of Zoology and Graduate Program in Ecology, University of Tennessee, Knoxville, Tennessee 37996, USA, and at the NERC Centre for Population Biology, Imperial College, Silwood Park, Ascot, Berks SL5 7PY, UK, John H. Lawton is at the NERC Centre for Population Biology, and Joel E. Cohen is at the Rockefeller University, New York, New York 10021, USA.

---

1. Darwin, C. *On the Origin of Species by Means of Natural Selection* (John Murray, London, 1859). (Facsimile reprint: Harvard University Press, Cambridge, MA, 1964).
2. Forbes, S. A. *Ecological Investigations of Stephen Alfred Forbes (1844–1932)* (reprint: Arno Press, New York, 1977).
3. Pierce, W. D., Cushman, R. A. & Hood, C. E. *U.S. Dept Agric. Bull.* **100,** 9–99 (1912).
4. Shelford, V. E. *Bull. Geogr. Soc. Chicago* **5** (1913) (reprint: Arno Press, New York, 1977).
5. Cohen, J. E. *Food Webs and Niche Space* (Princeton University Press, Princeton, NJ 1978).
6. Cohen, J. E., Briand, F. & Newman, C. M. *Community food webs: Data and theory* (Springer-Verlag, New York, 1990).
7. Cohen, J. E. *Ecologists Co-operative Web Bank* (*ECOWeB*™). *Version 1.0. Machine Readable Data Base of Food Webs* (Rockefeller University, New York, 1989).
8. Sugihara, G. *Proc. Symp. appl. Math.* **30,** 3–101 (1984).
9. Pimm, S. L. *Food Webs* (Chapman and Hall, London, 1982).
10. Paine, R. T. *Ecology* **69,** 1648–1654 (1988).
11. Jeffries, J. J. & Lawton, J. H. *Freshwater Biol.* **15,** 105–112 (1985).
12. Cohen, J. E. *Theor. Pop. Biol.* **37,** 55–90 (1990).
13. Sugihara, G., Schoenly, K. & Trombla, A. *Science* **245,** 48–52 (1989).
14. Warren, P. *Am. Nat.* **316,** 689–700 (1990).
15. Winemiller, K. O. *Am. Nat.* **134,** 960–968 (1989).
16. Beaver, R. A. *Ecol. Ent.* **10,** 241–248 (1985).
17. Kitching, R. L. & Pimm, S. L. *Proc. Ecol. Soc. Austr.* **14,** 123–139 (1986).
18. Kitching, R. L. & Beaver, R. A. in *Living in Patchy Environments* (eds Shorrocks, B. & Swingland, I. R.) 147–175 (Oxford University Press, Oxford, 1990).
19. Schoener, T. W. *Ecology* **70**, 1559–1589 (1989).
20. Kitching, R. L. *Oikos* **48,** 280–288 (1987).
21. Schoenly, K. & Cohen, J. E. *Ecol. Monogr.* (in the press).
22. Schoenly, K., Beaver, R. A. & Heumier, T. A. *Am. Nat.* (in the press).
23. Yodzis, P. *Oecologia* (*Berl.*) **65,** 86–88 (1984).
24. Pimm, S. L. & Kitching, R. L. *Oikos* **50,** 302–307 (1987).
25. Lawton, J. H. in *Ecological Concepts* (ed. Cherett, J. M.) 43–78 (Blackwell Scientific Publications, Oxford, 1989).
26. Warren, P. H. & Lawton, J. H. *Oecologia* (*Berl.*) **74,** 231–235 (1987).
27. Vézina, A. F. *Oecologia* (*Berl.*) **67,** 555–565 (1985).
28. Carpenter, S. R. *Complex Interactions in Lake Communities* (Springer-Verlag, New York, 1988).
29. Pimm, S. L. *The Balance of Nature?* (University of Chicago Press, Chicago, 1991).
30. Robinson, J. V. & Valentine, W. D. *J. theor. Biol.* **81,** 91–104 (1979).
31. Shigesada, N., Kawasaki, K. & Teramoto, E. *Theor. Pop. Biol.* **36**, 311–338 (1989).
32. Post, W. M. & Pimm, S. L. *Math. Biosci.* **64,** 169–192 (1983).
33. Drake, J. A. *J. theor. Biol.* **147,** 213–234 (1990).
34. Holt, R. D. *Theor. Pop. Biol.* **12,** 197–229 (1977).
35. Mithen, S. J. & Lawton, J. H. *Oecologia* (*Berl.*) **69,** 542–550 (1986).
36. Pimm, S. L. & Rice, J. A. *Theor. Pop. Biol.* **32,** 303–325 (1987).
37. Cohen, J. E., Luczak, T., Newman, C. M. & Zhou, Z. M. *Proc. R. Soc.* **B240,** 607–627 (1990).
38. May, R. M. *Nature* **238,** 413–414 (1972).
39. Pimm, S. L. *Nature* **307,** 321–326 (1984).
40. Redfearn, A. & Pimm, S. L. in *Insect Pests* (eds Barbosa, P. & Schultz, J. C.). 99–133 (Academic Press, Orlando, Fl, 1987).
41. Redfearn, A. & Pimm, S. L. *Ecol. Monogr.* **58,** 39–55 (1988).
42. Redfearn, A. & Pimm, S. L. in *Predation* (ed. Crawley, M.) (Blackwell Scientific Publications, Oxford, 1991).
43. Schoener, T. W. *Am. Nat.* **122,** 240–285 (1983).
44. Connell, J. H. *Am. Nat.* **122,** 661–696 (1983).
45. Sih, A., Crowley, P., McPeek, M., Petranka, J. & Strohmeier, K. *A. Rev. Ecol. Syst.* **16,** 269–311 (1985).
46. Yodzis, P. *Ecology* **69,** 508–515 (1988).
47. Patten, B. C. *Can. J. Fish. Aq. Sci.* **213,** 119–138 (1985).
48. Lane, P. A. in *The Biology of Mutualism: Ecology and Evolution* (ed. Boucher, D. H.) 345–374 (Croom Helm, London, 1985).
49. Brown, J. H., Davidson, D. W., Munger, J. C. & Inouye, R. S. in *Community Ecology* (eds Diamond, J. & Case, T. J.) 41–62 (Harper & Row, New York, 1986).
50. King, A. W. & Pimm, S. L. *Am. Nat.* **122,** 229–239 (1983).
51. McNaughton, S. J. *Ecol. Monogr.* **55,** 259–294 (1985).
52. Polis, G. *Am. Nat.* (in the press).
53. May, R. M. *Science* **241,** 1441–1449 (1988).
54. Hawkins, B. A. & Lawton, J. H. *Nature* **326,** 788–790 (1987).
55. Kohn, A. J. *Ecol. Monogr.* **29,** 47–90 (1959).

ACKNOWLEDGEMENTS. Authors are listed in reverse alphabetical order. We thank P. Warren for helpful suggestions. J.E.C. was partly supported by the NSF and by the hospitality of Mr and Mrs W. T. Golden. During the preparation of this paper S.L.P. was supported by the NERC Centre for Population Biology and the University of Tennessee; he also thanks J. K. Pimm.

Medium-range order in the cation distribution of a calcium silicate glass

P. H. Gaskell*, M. C. Eckersley*, A. C. Barnes†‡ & P. Chieux†

* University of Cambridge, Cavendish Laboratory, Madingley Road, Cambridge CB3 0HE, UK
† Institut Laue-Langevin, Avenue des Martyrs, 38042 Grenoble Cedex, France

Silicate glasses have conventionally been regarded as silicate frameworks in which cations are distributed at random. Neutron scattering from isotopically substituted samples allows correlations between cations to be investigated beyond the nearest-neighbour coordination shell, and shows that a degree of ordering persists over distances approaching 1 nm. The structural picture that emerges has elements of long-range randomness coexisting with both short- and medium-range order.

DEFINITION of the nature and extent of atomic ordering in simple amorphous solids is an important experimental and theoretical challenge. The structure of many diatomic oxide glasses, such as B_2O_3, SiO_2 and GeO_2, measured over a distance scale greater than ~2 nm is best represented as a disordered, isotropic solid but with considerable order on a scale of nearest-neighbour interatomic distances. For the alkali and alkaline-earth silicate glasses, the conventional (Zachariasen-Warren) model[1,2] suggests that the structure consists of a framework formed by SiO_4 tetrahedra, into the interstices of which cations such as calcium are generally thought to fit. These interstices were thought to be poorly defined, but it has recently become clear[3,4] that the first-neighbour shell is relatively ordered. The distribution of these 'network-modifying' atoms is, however, still an open question, a random distribution determined solely by macroscopic parameters such as composition and density being the general assumption. Here we present direct evidence that the distribution of calcium atoms in a calcium silicate glass is non-random, with features that reflect a more extensive structural organization bearing many of the characteristics of the crystalline form.

For the oxide glasses, typified by the silicate described here, the experimental challenge is to establish atomic distribution functions with sufficient precision and information content to generate and test structural models. In earlier work[4], we used neutron scattering with isotopic substitution of Ca in a silicate glass to obtain a weighted sum of the subset of partial pair-correlation functions centred on the calcium atom. This technique revealed a high degree of ordering in the immediate environment of Ca. Here we extend the technique to obtain a direct measurement of the Ca-Ca distribution, which provides strong evidence that such glasses are more extensively ordered than previously seemed possible.

The glass chosen for this investigation is identical to that studied earlier[4]: $(CaO)_{48.0}(SiO_2)_{49.0}(Al_2O_3)_{3.0}$. This is close to the metasilicate composition, allowing comparison with data for crystalline $CaSiO_3$ (wollastonite). It is free from sub-liquidus immiscibility, as shown by high-resolution transmission electron micrographs[5], and crystallization is suppressed by the addition of a small amount of Al_2O_3. We took care in melting and quenching to ensure that only the isotopic concentration of Ca differed in three specimens containing essentially pure natural Ca (^{nat}Ca), ^{44}Ca and an equal mixture of the two (^{mix}Ca). The scattered intensities were measured using the twin-axis diffractometer D4 at the Institut Laue-Langevin, Grenoble. We then obtained two first differences between the scattered intensities, $I(\text{nat}) - I(\text{mix})$ and $I(\text{mix}) - I(44)$.

The normalized scattering intensity is proportional to the differential scattering cross-section, $d\sigma/d\Omega$, corrected for absorption, multiple scattering and inelasticity:

$$\frac{1}{N}\frac{d\sigma}{d\Omega} = \frac{\sigma_s}{4\pi} + \sum_{\alpha\beta} c_\alpha c_\beta b_\alpha b_\beta (S_{\alpha\beta}(Q) - 1) \qquad (1)$$

Here N is the number of scattering atoms, σ_s is the sum of coherent and incoherent cross-sections, and c_α and b_α are atomic fractions and scattering lengths for atoms of species α. $S_{\alpha\beta}(Q)$ is a Faber-Ziman partial structure factor which contains information on the arrangement of β atoms around α, and $Q = 4\pi \sin\theta/\lambda$, where 2θ is the scattering angle and λ the neutron wavelength.

Subtraction of differential cross-sections for two isotopically substituted specimens cancels identical terms in $S_{\alpha\beta}(Q)$ for atom pairs that exclude Ca. Separating terms involving like atoms leads to:

$$\begin{aligned}\Delta_{Ca}(Q) &= c_{Ca}^2(b_{Ca}^2 - b''^2_{Ca})(S_{CaCa}(Q) - 1)\\ &\quad + 2\sum_{\beta\neq Ca} c_{Ca} c_\beta b_\beta \Delta b (S_{Ca\beta}(Q) - 1) \qquad (2)\end{aligned}$$

Here, $\Delta b = b_{Ca} - b''_{Ca}$, where b_{Ca} and b''_{Ca} are the scattering lengths for the two Ca isotopes and differences in the Q-independent term $\sigma_s/4\pi$ have been subtracted.

Previously[4] we reported a measurement of the first difference, $\Delta_{Ca}(Q)$, and analysis of the weighted pair correlation function $G_{Ca}(r)$ obtained by Fourier transformation:

$$\begin{aligned}G_{Ca}(r) &= 2/\pi \int_0^\infty Q\Delta_{Ca}(Q)\sin(Qr)\,dQ\\ &= c_{Ca}^2(b_{Ca}^2 - b''^2_{Ca})G_{CaCa}(r)\\ &\quad + 2\sum_{\beta\neq Ca} c_{Ca} c_\beta b_\beta \Delta b G_{Ca\beta}(r) \qquad (3)\end{aligned}$$

The functions $G_{\alpha\beta}(r)$ are defined by

$$G_{\alpha\beta}(r) = 4\pi r\rho_0(g_{\alpha\beta}(r) - 1) \qquad (4)$$

with $g_{\alpha\beta}(r) = 1 + (2\pi^2 r\rho_0)^{-1}\int_0^\infty Q(S_{\alpha\beta}(Q) - 1)\sin(Qr)\,dQ$, and ρ_0 the average atomic density.

For the glass considered here, $G_{Ca}(r)$ is dominated by the Ca-O distribution, especially at low values of r. The Ca-O first-neighbour distances centred on 0.237 nm can be extracted with considerable precision: about 85% of the 6.15 (±0.17) oxygen neighbours lie in a narrow distribution with a standard deviation of 0.012 nm, compared with an estimated 0.011 nm for the crystal. The remaining (0.95) oxygen atoms occupy a broad tail stretching from ~0.25 to 0.285 nm.

In c-$CaSiO_3$, Ca-Ca and Ca-Si distances are almost equal (0.36 and 0.34 nm respectively). In the glass, a pronounced second peak is seen at 0.35 nm, and the earlier indirect evidence

---

‡ Present address: H. H. Wills Physics Laboratory, University of Bristol, Royal Fort, Tyndall Avenue, Bristol BS8 1TL, UK.

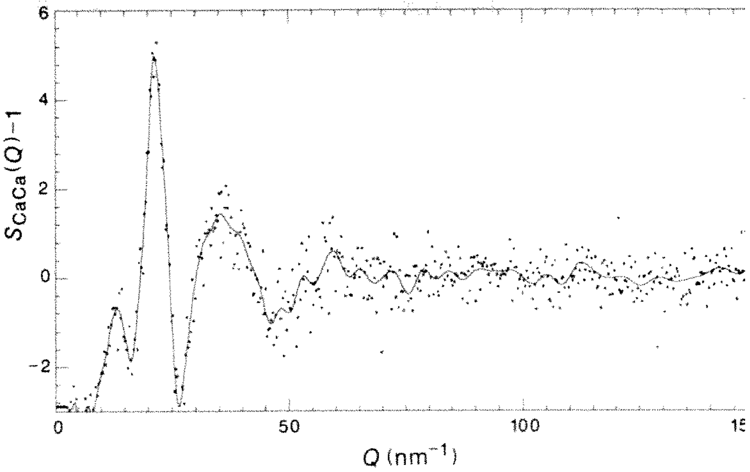


FIG. 1 Experimental double-difference data $S_{CaCa}(Q)$ for the calcium silicate glass. Points are raw data ; full line is a smoothed version obtained by fitting the differential cross-sections with a cubic spline before subtraction. The signal is significantly greater than the noise level only for $Q \leq 80$ nm^{-1}.

suggests that this feature does, in part, represent Ca–Ca first-neighbour distances. An alternative assumption of a random distribution of Ca atoms in the Si–O network leads to a larger mean separation, 0.44 nm.

Although this circumstantial evidence favours a Ca–Ca distance associated with a bonding scheme similar to that of the crystal, experimental first-difference data alone cannot discriminate unambiguously between the two possibilities. Nor can it be claimed that the first coordination sphere of about six oxygens is in an octahedral arrangement, and nothing can be said about the manner in which Ca-centred polyhedra are linked. In c-$CaSiO_3$, octahedra are linked at edges forming infinite trioctahedral Ca–O sheets. A direct measurement of the Ca–Ca pair correlation function is thus required to establish, firmly, whether randomness or a degree of medium-range ordering should form the pattern for future structural modelling of this and similar materials.

The isotopic concentrations of the three glasses were chosen such that Δb values corresponding to the first-difference data sets were identical. Subtraction of the two first differences then cancels terms other than those involving $S_{CaCa}(Q)$. Thus:

$$\Delta(\Delta_{Ca}(Q)) = c_{Ca}^2[\Delta_1(b_{Ca}^2) - \Delta_2(b_{Ca}^2)[S_{CaCa}(Q)-1] \qquad (5)$$

where $\Delta_1(b_{Ca}^2)$ and $\Delta_2(b_{Ca}^2)$ are differences in the squares of the scattering lengths for each pair of Ca isotopes.

Simplifying the expression for the double difference leads to:

$$\begin{gathered} 2\Delta(\Delta_{Ca}(Q)) = c_{Ca}^2(b_{Ca} - b''_{Ca})^2(S_{CaCa}(Q)-1) \quad \text{or} \\ S_{CaCa}(Q) = 1 + 2\Delta(\Delta_{Ca}(Q))/(c_{Ca}^2(b_{Ca} - b''_{Ca})^2) \end{gathered} \qquad (6)$$

where b and b'' are the scattering lengths for the two end members, and the scattering length of the intermediate composition, b'_{Ca}, is chosen so that $b_{Ca} - b'_{Ca} = b'_{Ca} - b''_{Ca}$.

A double-difference experiment thus gives direct information on the Ca–Ca distribution. The experimental problem, however, is that the signal is very weak and subject to sizeable systematic and random errors. The 'total' structural signal oscillates around the isotropic term $\sigma_s/4\pi$ (~25 fm^2) with an amplitude $\sum_{\alpha\beta} c_\alpha c_\beta b_\alpha b_\beta$ $(S_{\alpha\beta}(Q)-1)$, which is typically one-fifth of this value. Realistic counting times (~1 day for a 5-g specimen) limit the signal-to-noise ratio to ~0.2% for the total function. The double-difference signal, of order $c_{Ca}^2(b-b'')^2/2 = 0.18$ fm^2, thus carries a random noise of ~30%. Alternatively expressed, the double-difference signal is over 600 times smaller than $S_{CaCa}(Q)$. Systematic errors are even more serious. Errors in composition of ~0.5% lead to 'contamination' of the double-difference data by the much stronger Si–O, O–O and Ca–O correlations. Because specimen masses are limited to ~6 g by the expense of the ^{44}Ca isotope, reproducible melting to this level of accuracy is not guaranteed, and this is the chief limitation to accuracy at present.

Figure 1 shows $S_{CaCa}(Q)$, both as the original data an as a function obtained by subtraction of cubic spline fits 1/N dσ/dΩ. Some error in these data, ascribed to inadequa subtraction of contributions other than Ca–Ca pairs, is immed ately evident in oscillations of $S_{CaCa}(Q)-1$ below the limit −1.0. (It would not be unreasonable to normalize the data the limiting value.) The Fourier transforms of $Q(S_{CaCa}(Q)-1)$ $G_{CaCa}(r)$ for both sets of data are essentially identical, an results for the smoothed data are shown in Fig. 2. The first pea in $G_{CaCa}(r)$ at 0.23 nm is presumably due to incomplete remov of the first-difference signal. Examination of Fourier transfor truncated at different values of Q_{max} shows that a large contrib tion to the feature at 0.23 nm originates from oscillations $S_{CaCa}(Q)$ beyond 80 nm^{-1} where the double-difference signal vanishingly small. The other main features of $G_{CaCa}(r)$ a insensitive to Q_{max}, apart from inevitable loss of resolution. Th most important feature is a strong, well-defined peak at ~0.3 0.39 nm. If this is the first Ca–Ca distance, R_1, then correspo dence with first-neighbour Ca–Ca distances in c-$CaSiO_3$ 0.36 nm is excellent. Together with the indirect eviden obtained from first-difference data[4], this direct measureme indicates first Ca–Ca distances close to the values observed the crystalline phase. There is little support for a mean Ca–C distance near 0.44 nm as predicted by a random model.

The Ca–Ca coordination number is subject to considerab (and as yet unquantified) errors, but a fit to the data sugges that ~5.2 neighbours lie between 0.36 and 0.40 nm, with a furth atom in a broad shell at ~0.46 nm. In c-$CaSiO_3$, there are 4.

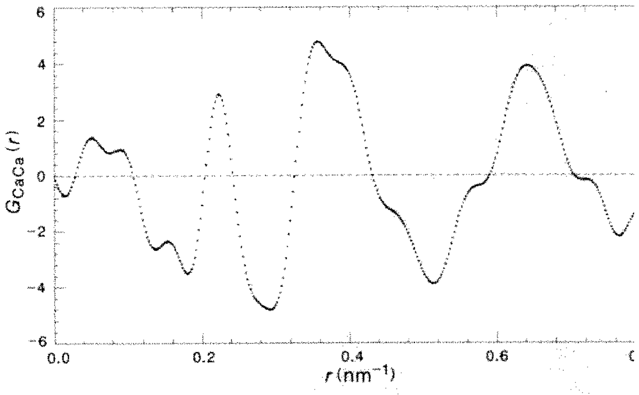


FIG. 2 Fourier transform of the double-difference data, $G_{CaCa}(r)$, obtain from smoothed $Q(S_{CaCa}(Q)-1)$ data truncated at $Q_{max} = 110$ nm^{-1}. Differe ces in $G_{CaCa}(r)$ introduced by smoothing are small. The strong peak ~0.38 nm probably corresponds to the nearest Ca–Ca distance and is clo to values found in c-$CaSiO_3$. The strong peak at 0.64 nm probably cor sponds to the second Ca–Ca neighbours. The first peak at ~0.23 nm thought to arise from inadequate subtraction of the first-difference sign (see text).

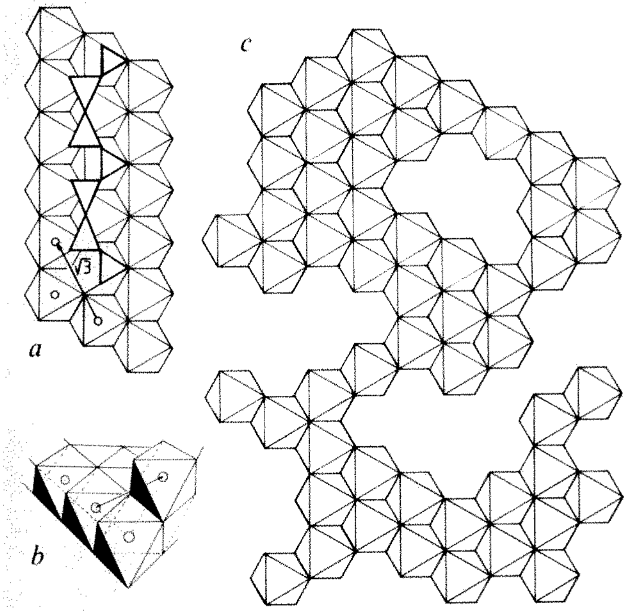


FIG. 3 *a*, Schematic view of trioctahedral strips of edge-sharing CaO_6 octahedra in c-$CaSiO_3$. A polymeric $(SiO_3)_\infty$ chain is shown between planes of close-packed oxygen anions. The arrow marks a Ca–Ca distance equal to $\sqrt{3}R_1$, where R_1 is the nearest-neighbour Ca–Ca distance. *b*, Corrugation of the sheet of edge-shared octahedra introduces distances such as that shown, in which the Ca–Ca distance (arrowed) is $\sqrt{2}R_1$. *c*, Part of a random sheet of edge-shared CaO_6 octahedra. The relative number of second and third neighbours (and so on) is related to the fractality of the sheet.

neighbouring Ca atoms in a broad distribution between 0.35 and 0.37 nm.

Features in the Ca–Ca distribution beyond 0.4 nm offer information on which new structural models can be based. One possibility, that the Ca–Ca distribution can be modelled in terms of dense-packed spheres, was considered and rejected for data on barium-containing glasses[6]. The idea was revived more recently[7] where peaks assigned to Cs–Cs pairs in caesium silicate glasses were shown to lie close to ratios of first, second and higher neighbour distances obtained in dense-packed models for monoatomic metals. Here we follow an alternative approach based on suggestions made previously[8,9,10]. The structures of crystalline pyroxenes, and pyroxenoids such as wollastonite, can be considered as a close-packed oxygen sub-lattice with cations such as Ca occupying octahedral interstices in alternate layers parallel to {111} (in a face-centred cubic model). Layers of Si atoms are interleaved, the silicons occupying tetrahedral interstices (Fig. 3*a*). It is arguable that this structural principle persists in glasses. A more controversial idea is that the need for polyvalent cations such as Ca to satisfy coordination requirements might be a dominant constraint, with Si–O chains suitably adapting their conformations to fit.

We propose as a working hypothesis that pyroxene and pyroxenoid glasses can be represented by distinguishable domains which are best described in terms of close-packing of oxygens. Sheets of interconnected CaO_6 octahedra are essentially parallel in these regions and are interleaved with linked SiO_4 tetrahedra. Defects can be described as corrugation and 'tearing' of the sheets, and variations in connectivity in the planes. In addition to such topological disorder, there will be geometrical distortions introduced by the topological 'defects'. Figure 3 shows these schematically.

The broad second peak centred on 0.64 nm observed in $G_{CaCa}(r)$ coincides with $\sqrt{3}R_1 = 0.65$ nm. The significance of this is that close-packed {111} planes of CaO_6 octahedra have second-neighbour Ca–Ca distances of $\sqrt{3}R_1$ (Fig. 3*a*). This additional information suggests that the 6.15 oxygen neighbours of Ca, for which it was impossible to assign any symmetry from first-difference data alone, form Ca-centred octahedra which are edge-linked in glasses as well as in crystals.

Almost as revealing as the presence of peaks in $G_{CaCa}(r)$ are their absences. A model based on randomly corrugated sheets of CaO_6 octahedra would involve a significant number of $\sqrt{2}R_1$ distances at 0.53 nm arising from the out-of-plane corrugations (Fig. 3*b*). This distance corresponds to a minimum in the experimental $G_{CaCa}(r)$ data, however, so this otherwise tempting model seems to be ruled out. Calcium-oxygen sheets must be essentially planar—topologically, if not geometrically.

A second significant absence is noted at $2R_1 = 0.75$ nm. A broad shoulder is seen in $G_{CaCa}(r)$ at this distance but is quite insignificant: the relative weakness of this feature may be related to disorder in the Ca–O sheet. Although on average this must be a trioctahedral sheet (valence and composition forbid significant deviations), disorder in the glass could be represented by random occupancy of octahedral interstices in a planar net. The ratio of $\sqrt{3}R_1$ and $2R_1$ distances then increases from 1.0 in the crystal to ~1.7 in the bifurcated sheet structure (Fig. 3*c*). The relative magnitude of the peaks at $\sqrt{3}R_1$ and $2R_1$ is thus a crude measure of the fractality of the Ca–O sheet, and the experimental data indicate large local deviations from the trioctahedrality.

Finally, the weaker correlations corresponding to distances in c-$CaSiO_3$ between Ca atoms separated by planes of Si–O tetrahedra, are also absent. These occur at distances ranging from 0.5 to 0.6 nm in the crystal, corresponding to a prominent minimum in the experimental curve for the glass. Again, fractal sheets of Ca and O atoms suggest the answer. Unless defects in adjacent sheets are correlated, which seems unlikely, interplanar distances cannot be well-defined.

The new information offered by this double-difference measurement has strengthened the evidence for ordered structures in silicate glasses, and possibly in other amorphous oxides. The indication from first-difference measurements that a Ca–Ca first-neighbour distance of 0.38 nm corresponds to a value typical of crystals is confirmed, and the peak in $G_{CaCa}(r)$ at 0.64 nm also indicates that the cation environment is octahedral, the octahedrons being edge-linked. The extent of sheet formation, and of deviations from this, can also be assessed from the absence of peaks in $G_{CaCa}(r)$ corresponding to the interplanar separations $\sqrt{2}R_1$ and $2R_1$. All these features are consistent with the theory that Ca lies in regions where the structure, although distorted, approximates to cubic close-packing. It would be difficult otherwise to understand the well defined local structure around a weakly bonded ion. As argued previously for metallic glasses[11], the presence of a well-defined local structure around such elements has wide implications for medium-range organization: In close-packed glasses (or in the close-packed regions of silicates and other oxide glasses) the occurrence of local ordering around highly-interconnected atoms such as Ca is a consequence of a well-developed medium-range structure. The evidence presented here goes some way to explore this territory. The double-difference technique has possible applications in structural studies of liquids, melts and disordered solids. Our more recent measurements include mixed Ca–Mg and Ca–Ni silicate and copper phosphate glasses. ☐

Received 22 November 1990; accepted 15 March 1991.

1. Zachariasen, W. H. *J. Am. chem. Soc.* **54**, 3841–3851 (1932).
2. Warren, B. E. & Biscoe, J. *J. Am. Ceram. Soc.* **21**, 259–265 (1938).
3. Greaves, G. N. in *Glass Science and Technology* **48** (eds Uhlmann, D. R. & Kreidl, N. J.) 1–76 (Academic, London, 1990).
4. Eckersley, M. C., Gaskell, P. H., Barnes, A. C. & Chieux, P. *Nature* **335**, 525–527 (1988).
5. Saeed, A. thesis, Univ. of Cambridge (1990).
6. Block, S. & Piermarini, G. J. *Phys. Chem. Glasses* **5**, 138–143 (1964).
7. Hanson, C. D. & Egami, T. *J. Non-cryst. Solids* **87**, 171–184 (1986).
8. Dent-Glasser, L. S. *Z. Kristallogr.* **149**, 291–305 (1979).
9. Liebau, F. *Structural Chemistry of Silicates* (Springer, Berlin, 1985).
10. Law, A. D. & Whittaker, E. J. W. *Mineralog. Mag.* **43**, 565–574 (1980).
11. Gaskell, P. H. *J. Non-cryst. Solids* **75**, 329–346 (1988).

ACKNOWLEDGEMENTS. We thank Pilkington plc (P.H.G.) and SERC (M.C.E.) for financial support, and P. Boden, D. R. Cockram, W. H. Flynn, H. L. McPhail, S. J. Monelly, G. W. F. Pardoe and B. Yale for help in glass melting and advice.

High-affinity NGF binding requires coexpression of the *trk* proto-oncogene and the low-affinity NGF receptor

Barbara L. Hempstead*, Dionisio Martin-Zanca†‡, David R. Kaplan§, Luis F. Parada‡ & Moses V. Chao*

* Hematology/Oncology Division, Department of Medicine, Department of Cell Biology and Anatomy, Cornell University Medical College,
1300 York Avenue, New York, New York 10021, USA
‡ Molecular Embryology Group, § Eukaryotic Signal Transduction Group, ABL-Basic Research Program,
NCI-Frederick Cancer Research & Development Center, PO Box B, Frederick, Maryland 21701, USA

Nerve growth factor (NGF) interacts with two different low-affinity receptors that can be distinguished by affinity crosslinking. Reconstitution experiments by membrane fusion and transient transfection into heterologous cells indicate that high-affinity NGF binding requires coexpression and binding to both the low-affinity NGF receptor and the tyrosine kinase *trk* gene product. These studies reveal a new growth factor receptor-mediated mechanism of cellular differentiation involving *trk* and the low-affinity NGF receptor.

CELL survival and differentiation in the nervous system are mediated by numerous growth factors, of which NGF has provided the most compelling evidence for a physiological role during development[1-3]. NGF is a selective neurotrophic factor for sympathetic and some sensory neurons of the peripheral nervous system, and for the ascending cholinergic neurons of the basal forebrain. Furthermore, administration of exogenous NGF can rescue developing neurons that would otherwise undergo naturally occurring cell death.

A key question regarding the action of NGF in responsive cells has been the molecular mechanism of signal transduction after ligand binding to the functional NGF receptor. The many effects of NGF on selective neuronal populations are directly dependent on initial binding of NGF to specific cell-surface receptors. Like other polypeptide growth factors, NGF interacts with responsive cells with biphasic equilibrium binding kinetics, reflecting two classes of receptors. Approximately 10–15% of the receptor sites display high-affinity binding (dissociation constant, $K_d = 10^{-11}$ M) and the remainder display low-affinity binding ($K_d = 10^{-9}$ M)[4-6]. The concentration of NGF necessary to elicit a response[7] and the detection of high-affinity NGF binding only in cells responsive to NGF[8-10] indicate that biological responsiveness to NGF depends on interactions with the high-affinity form of the receptor. No biological responses mediated solely by the low-affinity receptor have been observed[11,12]. These differences imply that the molecular events mediating the change in receptor affinity are involved in the signal transduction of NGF.

Complementary DNAs representing the NGF receptor messenger RNA have been isolated after gene transfer of genomic DNA and screening with monoclonal antibodies that either block or alter NGF binding in human melanoma cells[13] or rat PC12 cells[14]. The sequence of the cDNA for human[15], rat[16] and chicken NGF receptor[17,18] indicates that the receptor is a transmembrane protein of relative molecular mass 42,000 (M_r 42K), which is glycosylated to yield a protein of about 75K *in vitro* ($p75^{\mathrm{NGFR}}$).

Although the cysteine-rich extracellular region of $p75^{\mathrm{NGFR}}$ shares homology with the B-cell-activation molecule, CDw40 (ref. 19), the tumour-necrosis-factor receptor[20-22], and T-cell-activation molecule, OX40 (ref. 23), no significant homology exists for the cytoplasmic region, including any similarity to tyrosine or serine/threonine kinases. When expressed in many cell lines, $p75^{\mathrm{NGFR}}$ displays no functional responses following ligand binding[12], and generates only low-affinity NGF-binding sites with a K_d of 10^{-9} M (refs 16, 24). Generation of both high- and low-affinity sites is observed only when NGF receptor cDNAs is introduced into selective cell lines of neuronal origin[25-27]. These observations suggest that the low-affinity NGF receptor participates in the formation of the high-affinity receptor complex, but that other cell-specific molecules are required for the generation of the high-affinity receptor and for transmembrane signalling[25].

NGF has been shown to bind to the proto-oncogene *trk*, and induce autophosphorylation by the *trk*-encoded tyrosine kinase in PC12 cells[28,29]. The product of the proto-oncogene *trk* is a transmembrane glycoprotein that is selectively expressed in the developing nervous system. The identification of the proto-oncogene *trk* ($p140^{\mathrm{prototrk}}$) as an NGF receptor in PC12 cells suggests that $p140^{\mathrm{prototrk}}$ could participate in high-affinity NGF binding and be involved in mediating the specific actions of NGF. Here we demonstrate that $p140^{\mathrm{prototrk}}$ displays a different interaction with NGF than does $p75^{\mathrm{NGFR}}$, although both receptors bind with a similar low-affinity K_d. Furthermore, the coexpression of proto-oncogene $p140^{\mathrm{prototrk}}$ and $p75^{\mathrm{NGFR}}$ are required for the formation of high-affinity NGF-binding sites.

Crosslinking of NGF receptors

Affinity crosslinking of NGF-responsive cells with ^{125}I-labelled NGF has been extensively used to characterize receptors for NGF. When the hydrophilic reagent ethyldimethylaminopropyl carbodiimide (EDAC) is used, only one complex of M_r 90–100K is seen (Fig. 1). This complex has been observed in whole brain preparations[30,31], and a variety of cell types including Schwann cells, melanoma, pheochromocytoma and neuroblastoma cells[32-35]. The complex can also be immunoprecipitated with antibodies against NGF, as well as with monoclonal antibodies against the receptor; the complex therefore consists of NGF and the low-affinity NGF receptor, $p75^{\mathrm{NGFR}}$. Other crosslinking reagents, such as the photoactivatable agent *N*-hydroxylsuccinimidyl-4-azidobenzoate (HSAB), generate two crosslinked complexes of 100K and 158K (ref. 36). The 158K crosslinked complex is generated only in cells that have high-affinity binding sites, including pheochromocytoma cells and neurons. A variety of models have been proposed to address the relationship between the two crosslinked species, including one in which the

---

† Present address: Instituto de Microbiología Bioquimica, CSIC, Universidad de Salamanca, 37008 Salamanca, Spain.

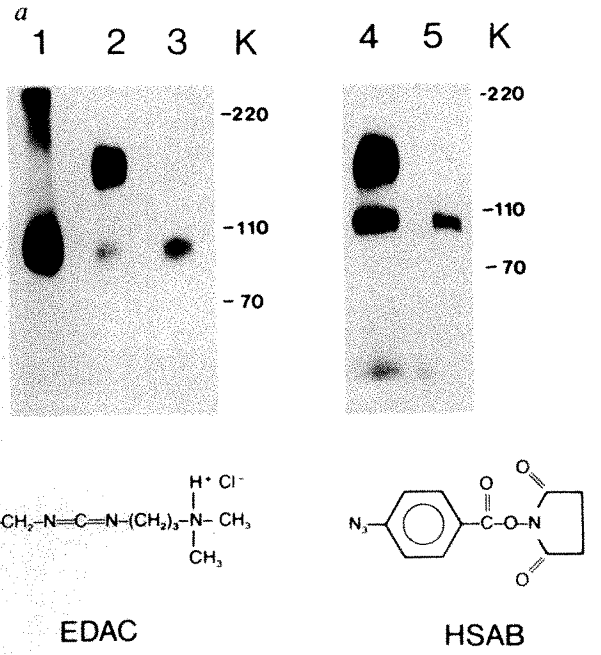


FIG. 1 *a*, Affinity crosslinking of ^{125}I-labelled NGF to pheochromocytoma PC12 cells. PC12 cells (2×10^6 per ml) were incubated with 0.5 nM ^{125}I-labelled NGF for 2 h at 4 °C and then crosslinked using EDAC[25] (lane 1) or HSAB[31,47] (lanes 2–5). Detergent extracts were immunoprecipitated as described[47] using anti-NGF antisera (Collaborative) (lanes 1, 2 and 4), IgG-192[14] antisera (lane 3), or anti-p75$^{\rm NGFR}$ antibody specific for amino acids 249–265 (lane 5). Immunoprecipitates were analysed by SDS-PAGE and autoradiography. About 5×10^6 cells were analysed in lane 1, and 30×10^6 cells were used in lanes 2–5. Exposure, 2 days at −70 °C. *b*, Structure of crosslinking reagents, EDAC and HSAB (Pierce).

158K species consists of a complex of p75$^{\rm NGFR}$ and a 60K protein[11,37].

Recent HSAB crosslinking has firmly identified the 158K species as p140$^{\rm prototrk}$ crosslinked to NGF[29]. This complex can be coimmunoprecipitated with p75$^{\rm NGFR}$ in PC12 cells with antibodies to mouse NGF (Fig. 1). However, three independently derived antibodies directed against different epitopes of p75$^{\rm NGFR}$ do not recognize the 158K crosslinked species. These antibodies are: the IgG-192 monoclonal antibody[14]; an antipeptide antibody specific for amino acids 249–265 (Fig. 1*a*, lane 5); an antibody specific for amino acids 163–396 of rat p75$^{\rm NGFR}$ (data not shown), a region encompassing 60 amino acids of the extracellular domain, the transmembrane domain and all of the cytoplasmic region. These experiments confirm that two distinct protein species can bind NGF, and support earlier biochemical data describing the different proteolytic susceptibility, pH sensitivity and Triton X-100 solubility of the two kinetic forms of the receptor[6,38–40].

An explanation for why the efficiency of crosslinking of p140$^{\rm prototrk}$ to ^{125}I-labelled NGF is different with EDAC than it is with HSAB (Fig. 1*b*) could lie in the mechanism of action of the crosslinking agents. EDAC is hydrophilic and directly crosslinks proteins by using a free carboxylic acid group; HSAB is hydrophobic, interacts with amino groups, and introduces an 8 Å spacer arm between the crosslinked proteins. Because EDAC does not crosslink NGF with p140$^{\rm prototrk}$ on PC12 cells, different protein–protein contacts are probably made between NGF and each of the two receptors. Indeed, the extracellular sequences of the human *trk* proto-oncogene product[41] and human p75 receptor[15] have very little amino-acid similarity, particularly in the pattern of their cysteine residues.

These crosslinking experiments illustrate fundamental differences between the interaction of NGF with p75$^{\rm NGFR}$ and its interaction with p140$^{\rm prototrk}$. The two species defined by HSAB crosslinking also differ in their phosphorylation, with the 100K species primarily having phosphorylated serine and threonine residues[42,43] and the 158K species containing phosphotyrosine[28,44]. Further, the high- and low-affinity forms of the receptor seem to differ in their cytoskeletal interactions[6,38] and in their ability to undergo ligand mediated internalization[37,45,46].

Membrane fusion experiments

Previous experiments using biochemical crosslinking of p75$^{\rm NGFR}$ to ^{125}I-labelled NGF demonstrated that the 100K crosslinked species is common to both the high- and low-affinity forms of the NGF receptor[34]. This model, in which p75$^{\rm NGFR}$ plus additional cellular components is required to produce the high-affinity form of the receptor, has been supported by gene transfer experiments. In these studies, a cDNA encoding the human p75$^{\rm NGFR}$ was introduced into cell lines of neuronal origin that lack the receptor. The resulting transfectants displayed both high- and low-affinity sites[25,27], the 100K and 158K crosslinked species[47], and responded to NGF[25–27]. Because p140$^{\rm prototrk}$ crosslinked to NGF is the 158K species, we tested whether p140$^{\rm prototrk}$ is directly involved in the conversion of low-affinity to high-affinity NGF binding by performing membrane fusion experiments.

A 3T3 transformant line overexpressing rat p140$^{\rm prototrk}$ (rtrk-3T3) binds ^{125}I-labelled NGF with low affinity[29]. Membranes isolated from this line were fused using polyethylene glycol (PEG)[48] to membranes from a fibroblast line (PAE1c) expressing p75$^{\rm NGFR}$, and the final membrane suspension was incubated with increasing concentrations of ^{125}I-labelled NGF. As previously demonstrated, PAE1c cells express receptors with only low-affinity binding sites[25]. Similarly, membranes isolated from rtrk-3T3 cells had an equilibrium binding constant consistent with low-affinity binding[29]. However, fusion of membranes from

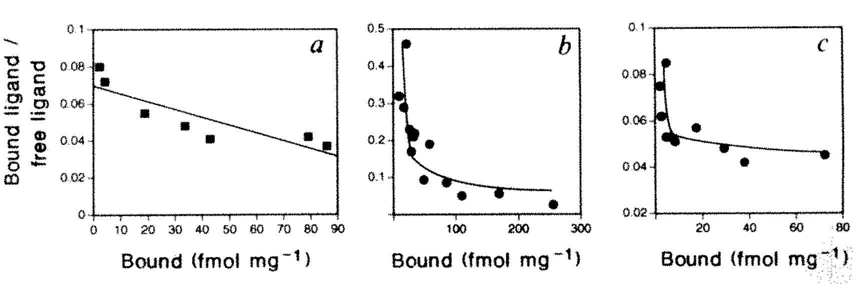


FIG. 2 ^{125}I-labelled NGF equilibrium binding analysis of fused membranes. Crude membrane preparations were isolated as described[25] from PAE1c and PAB9 cells (PA317 fibroblasts expressing p75$^{\rm NGFR}$)[25,47], mouse 3T3 fibroblasts, rtrk-3T3 (ref. 29) and NR18 cells[51]. Binding data were plotted according to Scatchard[49]. *a*, PAE1c membranes (1.32 mg) fused with 3T3 membranes (2.3 mg). *b*, PAE1c membranes (4 mg) fused with rtrk-3T3 membranes (0.40 mg). *c*, NR18 membranes (2.25 mg) fused with PAB9 membranes (2.2 mg). METHODS. Membrane preparations were fused using PEG according to Robb[48], and equilibrium binding analysed by vacuum filtration as described[25]. Each binding reaction was carried out in triplicate, with and without a thousand-fold excess of unlabelled NGF. Binding data was corrected for nonspecific binding by subtracting values obtained in the presence of unlabelled NGF. Specific binding ranged from 60 to 90% of total binding. Amount of membrane protein used for each reaction was 30 μg. ^{125}I-labelled NGF was prepared by lactoperoxidase treatment.

PAE1c cells and rtrk-3T3 cells produced both high- and low-affinity ^{125}I-labelled NGF binding by Scatchard[49] analysis (Fig. 2*b*). Using the LIGAND program[50], two site analysis and curve fitting generated equilibrium dissociation constants of 2.5×10^{-11} M and 1.4×10^{-9} M, consistent with values obtained in ^{125}I-labelled NGF binding experiments with sensory neurons and cultured PC12 cells[4,6]. The generation of the biphasic curve was the direct consequence of fusion of p140$^{\mathrm{prototrk}}$-containing membranes with PAE1c membranes, because fusion of membranes from untransfected 3T3 cells with PAE1c membranes (Fig. 2*a*) produced a linear Scatchard plot ($K_d = 1.5 \times 10^{-9}$ M), reflecting binding to low-affinity NGF receptors in PAE1c cells.

This experiment supports the previous gene transfer experiments suggesting that additional cell-specific molecules are required for high-affinity binding. One of those experiments used the pheochromocytoma cell line NR18 that lacks p75$^{\mathrm{NGFR}}$ as the recipient cell. These cells have been described as incapable of binding NGF[51], implying that p140$^{\mathrm{prototrk}}$ is not expressed in them. But RNA blot analysis indicates that NR18 cells express *trk* messenger RNA, but at very low levels compared with PC12 cells (Fig. 3). In addition, HSAB crosslinking reveals extremely low amounts of p140$^{\mathrm{prototrk}}$ in NR18 cells (data not shown).

To verify that NR18 cells can reconstitute high-affinity binding, PEG-mediated membrane fusion experiments were carried out with fibroblast cells expressing p75$^{\mathrm{NGFR}}$ and NR18 cells. NR18 membranes did not detectably bind NGF, as determined by filter binding, presumably owing to the low numbers of p140$^{\mathrm{prototrk}}$ molecules in this mutant cell line. However, fusion of NR18 membranes with membranes containing p75$^{\mathrm{NGFR}}$ (Fig. 2*c*) yielded both high- and low-affinity classes of receptors ($K_d = 2.5 \times 10^{-11}$ M and 1.4×10^{-9} M). The much lower number of high-affinity sites (300 sites per cell) reconstituted after membrane fusion is consistent with the low level of *trk* expression in this mutant PC12 cell line. These experiments also indicate that in the absence of p140$^{\mathrm{prototrk}}$ receptors (Fig. 2*a*), no high-affinity binding could be detected. Furthermore, fusion of membranes with PEG is necessary to generate the biphasic Scatchard plot, because omission of PEG during the procedure leads t only low-affinity binding (data not shown).

Transient transfection of *trk*

The membrane fusion experiments directly implicate p140$^{\mathrm{prototr}}$ as the protein that participates in high-affinity NGF binding i membrane preparations. To test whether p140$^{\mathrm{prototrk}}$ is involve in the formation of high-affinity sites by transfection, COS- cells and human melanoma cells were used in transien expression experiments. The full-length cDNA encoding rat *tr* and a 1.5-kilobase (kb) cDNA representing the full length o the coding region of the human p75$^{\mathrm{NGFR}}$ were subcloned int the plasmid pCMV5 vector[52].

COS cells were transfected with each plasmid alone and als with both together at a ratio of 1 to 10 (*trk*: p75$^{\mathrm{NGFR}}$). CO cells do not normally express *trk* mRNA or p140$^{\mathrm{prototrk}}$ (Figs and 4), but 48 hours after transfection, abundant *trk* mRN and p140$^{\mathrm{prototrk}}$ are produced. The appearance of the receptor at the cell surface and their ability to interact with NGF wa verified by affinity crosslinking. HSAB crosslinking of ^{125}I labelled NGF to COS cells transfected with the *trk* cDNA yield a 158K species, whereas affinity crosslinking of COS cell expressing p75$^{\mathrm{NGFR}}$ yields a 90K crosslinked species. When th two plasmids were used in transient transfectants, both the 158K and 90K crosslinked species were generated (Fig. 4, lane 7). Crosslinked p140$^{\mathrm{prototrk}}$ was also recognized specifically using anti-Trk antibodies (lane 8), whereas untransfected COS cells did not display any crosslinked species when either reagent was used. The relative lack of crosslinking of p140$^{\mathrm{prototrk}}$ by EDAC (Fig. 4, lane 5) confirms that the interaction of NGF with p140$^{\mathrm{prototrk}}$ in PC12 cells is recapitulated in transfected COS cells.

Crude membranes were isolated from transfected COS cells and assayed by filter binding using ^{125}I-labelled NGF. Membranes prepared from cells expressing p140$^{\mathrm{prototrk}}$ alone generated a linear Scatchard plot (Fig. 5*a*), with a K_d of 3.3×10^{-9} M, consistent with the equilibrium binding constant measured in

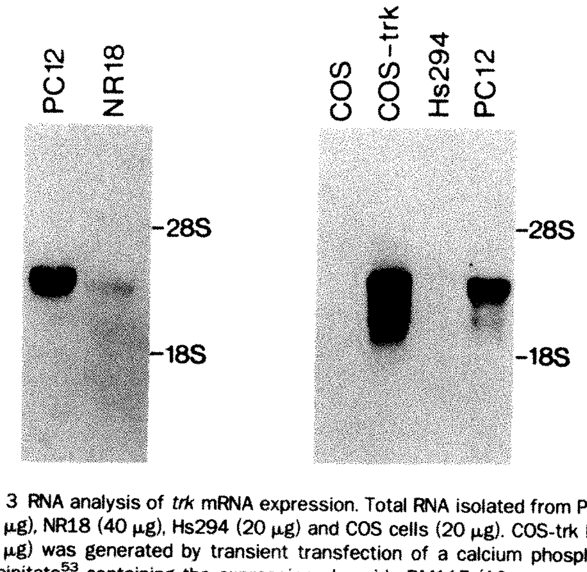


FIG. 3 RNA analysis of *trk* mRNA expression. Total RNA isolated from PC12 (40 μg), NR18 (40 μg), Hs294 (20 μg) and COS cells (20 μg). COS-trk RNA (20 μg) was generated by transient transfection of a calcium phosphate precipitate[53] containing the expression plasmid pDM115 (10 μg per dish) for the rat *trk* cDNA. After overnight incubation, the cells were treated with 20% glycerol for 1 min. The slightly longer mRNA for the transfected *trk* arises because of an upstream start site in the expression plasmid. Migration positions of ribosomal RNA markers are shown on the right.
METHODS. RNA was isolated from confluent cells and separated by electrophoresis in a 1% agarose gel containing 2.2 M formaldehyde. Expression of *trk* mRNA was assessed by hybridization to a 464-base-pair insert from pDM97, specific for rat *trk*. The probe was radiolabelled by primer extension and hybridized at 50% formamide, 5× SSCPE, 1× Denhardt's solution, salmon sperm DNA (100 μg ml^{-1}) and 10% dextran sulphate. Filters were washed to 0.5× SSC at 60 °C followed by autoradiography.

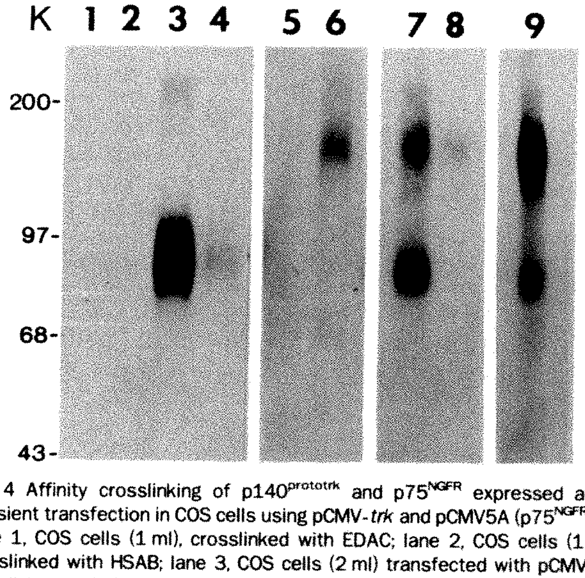


FIG. 4 Affinity crosslinking of p140$^{\mathrm{prototrk}}$ and p75$^{\mathrm{NGFR}}$ expressed after transient transfection in COS cells using pCMV-*trk* and pCMV5A (p75$^{\mathrm{NGFR}}$)[15]. Lane 1, COS cells (1 ml), crosslinked with EDAC; lane 2, COS cells (1 ml) crosslinked with HSAB; lane 3, COS cells (2 ml) transfected with pCMV5A, crosslinked with EDAC; lane 4, COS cells (2 ml) transfected with pCMV5A, crosslinked with HSAB: lane 5, COS cells (2 ml) transfected with pCMV-trk, crosslinked with EDAC; lane 6, COS cells (2 ml) transfected with pCMV-trk, crosslinked with HSAB; lane 7, COS cells (4.5 ml) transfected with 4 μg pCMV-trk and 10 μg pCMV5A per dish, crosslinked with HSAB; lane 8, same as lane 7 except immunoprecipitation was carried out with anti-Trk antisera; and lane 9, PC12 cells (1 ml) crosslinked with HSAB.
METHODS. Cells (2×10^6 per ml) were incubated with 1 nM ^{125}I-labelled NGF for 2 h at 4 °C, followed by crosslinking as described previously[47]. After detergent lysis, extracts were immunoprecipitated with anti-NGF antisera (lanes 1–7 and 9) or anti-p140$^{\mathrm{prototrk}}$ (lane 8), washed extensively, and subjected to SDS-PAGE.

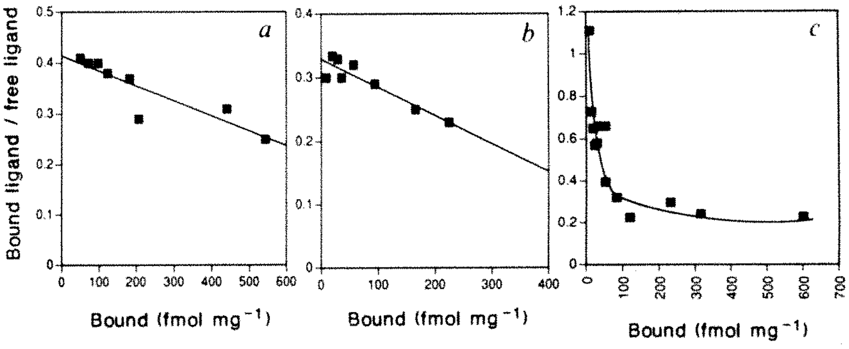


FIG. 5 Equilibrium binding analysis of transfected receptors expressed in COS cells. COS cells were transiently transfected, and collected after 60 h for crude membrane preparation and binding with ^{125}I-labelled NGF. Equilibrium binding analysis was performed as described in Fig. 2. *a*, COS cells transfected with pDM115 ($p140^{prototrk}$). *b*, COS cells transfected with pCMV5A ($p75^{NGFR}$). *c*, COS cells transfected with pDM115 and pCMV5A at a 1 to 10 ratio.

membranes from stably transfected 3T3 cells[29]. Although these results do not exclude the presence of a few higher-affinity binding sites, computer analysis of many different COS cell transfectants and fibroblast lines have yielded equivalent K_d values. COS cells expressing $p75^{NGFR}$ also generated a linear Scatchard plot, with a K_d of 1.1×10^{-9} M (Fig. 5*b*). These equilibrium binding constants are consistent with earlier studies of $p75^{NGFR}$ expression in heterologous cells[16,24,25]. Immunofluorescence of the transfected cells indicates that at least 30–40% of the COS cells express $p75^{NGFR}$. When membrane preparations from cells transfected with both *trk* and NGF receptor cDNAs were examined, both high- and low-affinity sites with a K_d of 3×10^{-11} M and 5×10^{-9} M, respectively, were found, in close agreement with the values obtained by PEG-mediated fusion of fibroblast membranes. Because these gene transfer experiments used the same type of cells and the same transfection protocol and equilibrium binding conditions, expression of both cDNAs appears to be required for high-affinity binding.

To confirm that coexpression of the $p75^{NGFR}$ and the $p140^{prototrk}$ receptors are required to reconstitute high-affinity binding sites, the *trk* cDNA was transiently expressed in the human Hs294 melanoma cell line, which expresses many p75 receptors[54], but no *trk* mRNA (Fig. 3). Hs294 cells express $p75^{NGFR}$ at the cell surface and display only low-affinity sites with a K_d of 5.9×10^{-9} M (Fig. 6*a*). Addition of NGF to these cells does not result in any of the responses associated with NGF treatment of PC12 cells. But when Hs294 cells were transfected with the *trk* expression plasmid pDM115, binding to ^{125}I-labelled NGF yielded a curvilinear Scatchard plot, indicating a high-affinity site with a K_d of 3.1×10^{-11} M and a lower-affinity site with a K_d of 5.5×10^{-9} M (Fig. 6*b*). Hence, low-affinity NGF binding can be converted to high-affinity binding with the expression of *trk* in transfected melanoma cells.

Discussion and implications

The molecular basis for high- and low-affinity NGF receptors has been an enigma. The results reported here provide strong support for the hypothesis that the high-affinity NGF binding site is composed of at least two NGF-binding components: the *trk* gene product and $p75^{NGFR}$, each of which binds NGF with a low-affinity K_d. This conclusion is supported by three experiments: (1) membrane fusion studies using cells independently expressing $p75^{NGFR}$ and $p140^{prototrk}$; (2) gene transfer experiments in which the $p75^{NGFR}$ and $p140^{prototrk}$ receptors are coexpressed in a cell line that normally expresses neither; and (3) gene transfer of a *trk* cDNA in cells of neuronal origin which have high levels of endogenous expression of $p75^{NGFR}$.

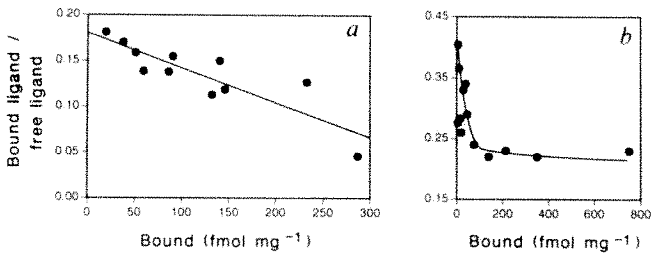


FIG. 6 Equilibrium binding analysis of HS294 melanoma cells expressing $p140^{prototrk}$. Hs294 melanoma cells[54] were transiently transfected with pDM115 using calcium phosphate[53], glycerol-shocked and collected for membrane preparation 60 h later. NGF binding was performed on membranes by filter binding[25]. Membranes were isolated from untransfected Hs294 cells (*a*) and Hs294 cells transfected with pDM115 (*b*).

Previous investigations of the molecular characteristics of the high-affinity and low-affinity receptor classes have not clearly defined the two species. Initial studies used affinity crosslinking of radiolabelled NGF to cells which express both high- and low-affinity receptors. Using HSAB[55], two complexes of 158K and 100K were detected[36]. In contrast, HSAB crosslinking of cells that express only the low-affinity NGF receptor yields only the 100K crosslinked species. These results led to the conclusion that the 158K species represents the high-affinity receptor crosslinked to NGF, and that the 100K species represents the low-affinity receptor crosslinked to NGF. Further evidence demonstrating that the two species differ in their trypsin sensitivity, Triton X-100 solubility, dissociation rate[5,6], pattern of phosphorylation[28,42–44], and lack of crossreactivity in immunoprecipitation experiments, strongly suggested that the two binding sites are represented by two independent protein moieties.

Other biochemical studies, however, suggested that the high-affinity receptor had features in common with the low-affinity receptor. When affinity crosslinking was performed using EDAC, only the 100K crosslinked complex was seen in PC12 cells bearing both high- and low-affinity receptors over a wide range of NGF concentration[34]. Gene transfer experiments further supported the idea that $p75^{NGFR}$ participates in the formation of the high-affinity receptor complex. Introduction of $p75^{NGFR}$ into appropriate cell lines resulted in the reconstitution of both high- and low-affinity sites, responsiveness to NGF treatment[25,26,56], and the appropriate HSAB crosslinking pattern (both 158K and 100K species)[47]. These results suggest that in addition to the low-affinity NGF receptor, another protein is required for formation of the high-affinity site.

The results of the study described here indicate that both $p140^{prototrk}$ and $p75^{NGFR}$ are required to form the high-affinity ligand-binding site. Membrane fusion studies imply that $p75^{NGFR}$ and $p140^{prototrk}$ receptor proteins do not need to be synthesized concomitantly to form high-affinity binding sites. In addition, reconstitution of high-affinity ligand binding by cotransfection of COS cells suggests that the appropriate translation and post-translational modification of the two proteins, and their migration to the cell surface to form the high-affinity site, do not necessarily require a neuronal cell environment.

Although signal transduction by NGF is likely to be initiated by tyrosine phosphorylation by $p140^{prototrk}$, the precise mechanisms that regulate ligand affinity remain unclear. Given the requirement for both $p75^{NGFR}$ and $p140^{prototrk}$, a model of high affinity receptor site formation can be proposed (Fig. 7). From affinity-crosslinking and equilibrium-binding studies, it is known that both proteins can interact with NGF, although it is uncertain whether they interact with a monomer or dimer of NGF. Analysis of equilibrium binding of ^{125}I-labelled NGF to $p140^{prototrk}$ clearly argues against $p140^{prototrk}$ alone being the high-affinity

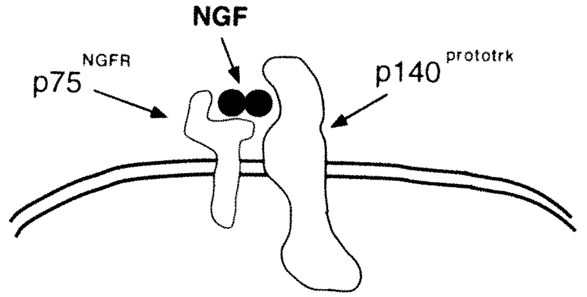


FIG. 7 Hypothetical model for high-affinity NGF binding. NGF exists as a noncovalent β dimer that can bind both to p75^{NGFR} and p140^{prototrk}. Previous experiments indicated that p140^{prototrk} alone can bind specifically to NGF[29]. Reconstitution of high-affinity binding after membrane fusion and transfection of *trk* in heterologous cells supports a model in which both p75^{NGFR} and p140^{prototrk} are required.

NGF receptor, when p140^{prototrk} is expressed independently of the low-affinity NGF receptor.

The binding studies do not address the role of p75^{NGFR} in directly modulating signal transduction. However, in previous studies, cells expressing mutant p75^{NGFR} lacking portions of the highly conserved p75 cytoplasmic domain fail to generate high-affinity sites when expressed in NR18 cells[47]. In addition, NR18 cells expressing these mutants lack normal tyrosine phosphorylation of cellular proteins in response to NGF[57]. These studies suggest that the cytoplasmic domain of p75^{NGFR} may interact with p140^{prototrk}, and that this interaction leads not only to the generation of a high-affinity site, but also to appropriate transmembrane signalling, as detected by tyrosine phosphorylation. Further experiments with chimaeric receptors containing cytoplasmic portions of the p75 molecule also support the role of p75^{NGFR} in signal transduction by NGF[58].

Our reconstitution experiments do not exclude the possibility of additional components in the fully functional high-affinity NGF receptor. Among the examples of multisubunit receptors are the interleukin-2 (IL-2) receptor with at least two subunits (α and β) directly interacting with IL-2 (ref. 59), and four other proteins associated with the receptor complex[60-62], and the interleukin-6 (IL-6) receptor, with an IL-6 binding subunit and a nonbinding associated protein, gp130, which acts in signal transduction[63]. In addition, heparin-like molecules are required for high-affinity binding of basic fibroblast growth factor to its receptor[64].

Intracellular molecules may also couple with the high-affinity NGF receptor complex after ligand activation. As has been previously demonstrated with the receptors for platelet-derived growth factor[65,66] and epidermal growth factor[67], and for the CD4 and CD8 antigens[68], the cytoplasmic domains of these transmembrane receptors can interact with a variety of soluble enzymes, including GAP, phospholipase C-γ and p56^{lck}, when the appropriate receptor is activated after ligand binding. From the model presented above, such protein–protein interactions could occur with the *trk* protein alone, p75^{NGFR} alone, or both species together.

Elucidation of the structural components of the functional high-affinity receptor is not only crucial in defining the mechanism of action of NGF, but will provide insight into the mode of signal transduction of other related neurotrophic factors. Brain derived neurotrophic factor (BDNF)[69] and neurotrophin-3 (NT-3) share significant homology with NGF[70,71] and act in a similar manner *in vitro*, enhancing neuronal survival and promoting cellular differentiation[72-75]. BDNF and NT-3 differ from NGF in that discrete subpopulations of neurons that are unresponsive to NGF, such as placode-derived nodose sensory neurons, respond to BDNF and NT-3 (refs 72, 76). Also, the developmental regulation and sites of synthesis of BDNF and NT-3 are distinct from those described for NGF[77,78].

BDNF binds to sensory neurons in a saturable and specific manner to high- and low-affinity sites[79]. Furthermore, BDN can recognize and bind to p75^{NGFR} expressed in fibroblast cell with kinetics similar to those of low-affinity binding[80]. NT- appears to bind to PC12 cells, but does not induce a differentiated phenotype[75], suggesting that the high-affinity NGF recepto complex can discriminate between NT-3 and its appropriat ligand NGF. Indeed, p75^{NGFR} could bind many different members of the neurotrophin family, and therefore can be considere to be a neurotrophin receptor. Because related *trk* tyrosin kinases such as *trkB* are expressed in distinct regions of th nervous system[81-83], specificity of neurotrophic action is mos probably generated by the expression of an appropriate *tr* tyrosine kinase. The identification of p140^{prototrk} as an NG receptor that participates as a subunit of the high-affinity NGF binding site will not only further our knowledge about th mechanism of signal transduction for NGF, but will also serv as a model receptor system for discriminating the actions o many different neurotrophic factors.

Received 11 March; accepted 4 April 1991.

1. Levi-Montalcini, R. & Angeletti, P. U. *Physiol. Rev.* **48,** 534–569 (1968).
2. Thoenen, H. & Barde, Y-A. *Physiol. Rev.* **60,** 1284–1335 (1980).
3. Levi-Montalcini, R. *Science* **237,** 1154–1164 (1987).
4. Sutter, A., Riopelle, R. J., Harris-Warrick, R. M. & Shooter, E. M. *J. biol. Chem.* **254,** 5972–598 (1979).
5. Landreth, G. E. & Shooter, E. M. *Proc. natn. Acad. Sci. U.S.A.* **77,** 4751–4755 (1980).
6. Schechter, A. L. & Bothwell, M. A. *Cell* **24,** 867–874 (1981).
7. Greene, L. A. *Brain Res.* **133,** 350–353 (1977).
8. Sonnenfeld, K. H. & Ishii, D. N. *J. Neurosci. Res.* **8,** 375–391 (1982).
9. Sonnenfeld, K. H. & Ishii, D. N. *J. Neurosci.* **5,** 1717–1728 (1985).
10. Green, S. H., Rydel, R. E., Connolly, J. L. & Greene, L. A. *J. Cell Biol.* **102,** 830–843 (1986).
11. Johnson, E. M. & Taniuchi, M. *Biochem. Pharmac.* **36,** 4189–4195 (1987).
12. Hempstead, B. L., Patil, N., Olson, K. & Chao, M. *Cold Spring Harb. Symp. quant. Biol.* **53,** 477–48 (1988).
13. Ross, A. H. *et al. Proc. natn. Acad. Sci. U.S.A.* **81,** 6681–6685 (1984).
14. Chandler, C. E., Parsons, L. M., Hosang, M. & Shooter, E. M. *J. biol. Chem.* **259,** 6882–6889 (1984
15. Johnson, D. *et al. Cell* **47,** 545–554 (1986).
16. Radeke, M. J. *et al. Nature* **325,** 593–597 (1987).
17. Large, T. H. *et al. Neuron* **2,** 1123–1134 (1989).
18. Heuer, J. G., Fatemie-Nainie, S., Wheeler, E. F. & Bothwell, M. *Devl Biol.* **137,** 287–304 (1990).
19. Stamenkovic, II, Clark, E. A. & Seed, B. *EMBO J.* **8,** 1403–1410 (1989).
20. Loetscher, H. *et al. Cell* **61,** 352–359 (1990).
21. Schall, T. J. *et al. Cell* **61,** 361–370 (1990).
22. Smith, C. A. *et al. Science* **248,** 1019–1023 (1990).
23. Mallet, S., Fossum, S. & Barclay, A. N. *EMBO J.* **9,** 1063–1068 (1990).
24. Chao, M. V. *et al. Science* **232,** 418–421 (1986).
25. Hempstead, B. L., Schleifer, L. S. & Chao, M. V. *Science* **243,** 373–375 (1989).
26. Matsushima, H. & Bogenmann, E. *Molec. cell. Biol.* **10,** 5015–5020 (1990).
27. Pleasure, S. J. *et al. Proc. natn. Acad. Sci. U.S.A.* **87,** 8496–8500 (1990).
28. Kaplan, D. R., Martin-Zanca, D. & Parada, L. F. *Nature* **350,** 158–160 (1991).
29. Kaplan, D. R., Hempstead, B. L., Martin-Zanca, D., Chao, M. V. & Parada, L. F. *Science* (in the press
30. Taniuchi, M., Schweitzer, J. B. & Johnson, E. M. *Proc. natn. Acad. Sci. U.S.A.* **83,** 1950–1954 (1986
31. Escandon, E. & Chao, M. V. *Devl Biol.* **142,** 293–300 (1990).
32. Grob, P. M., Berlot, C. H. & Bothwell, M. A. *Proc. natn. Acad. Sci. U.S.A.* **80,** 6819–6823 (1983
33. Taniuchi, M., Clark, H. B. & Johnson, E. M. *Proc. natn. Acad. Sci. U.S.A.* **83,** 4094–4098 (1986).
34. Green, S. H. & Greene, L. A. *J. biol. Chem.* **261,** 15316–15326 (1986).
35. Hempstead, B. L. & Chao, M. V. *Recent Prog. Horm. Res.* **45,** 441–466 (1989).
36. Hosang, M. & Shooter, E. M. *J. biol. Chem.* **260,** 655–662 (1985).
37. Hosang, M. & Shooter, E. M. *EMBO J.* **6,** 1197–1202 (1987).
38. Vale, R. D. & Shooter, E. M. *J. Cell Biol.* **94,** 710–717 (1982).
39. Block, T. & Bothwell, M. *J. Neurochem.* **40,** 1654–1663 (1983).
40. Buxser, S. E., Watson, L. & Johnson, G. L. *J. cell Biochem.* **22,** 219–233 (1983).
41. Martin-Zanca, D. *et al. Molec. cell. Biol.* **9,** 24–33 (1989).
42. Grob, P. M., Ross, A. H., Koprowski, H. & Bothwell, M. A. *J. biol. Chem.* **260,** 8044–8049 (1985
43. Taniuchi, M., Johnson, E. M., Roach, P. J. & Lawrence, J. C. *J. biol. Chem.* **261,** 13342–13349 (1986
44. Meakin, S. O. & Shooter, E. M. *Neuron* **6,** 153–163 (1991).
45. Olender, E. J. & Stach, R. W. *J. biol. Chem.* **255,** 9338–9343 (1980).
46. Bernd, P. & Greene, L. A. *J. biol. Chem.* **259,** 15509–15516 (1984).
47. Hempstead, B. L., Patil, N., Thiel, B. & Chao, M. V. *J. biol. Chem.* **265,** 9595–9598 (1990).
48. Robb, R. J. *Proc. natn. Acad. Sci. U.S.A.* **83,** 3992–3996 (1986).
49. Scatchard, G. *Ann. N.Y. Acad. Sci.* **52,** 660–672 (1949).
50. Munson, P. J. & Rodbard, D. *Analyt. Biochem.* **107,** 220–239 (1980).
51. Bothwell, M. A., Schechter, A. L. & Vaughn, K. M. *Cell* **21,** 857–866 (1980).
52. Andersson, S. *et al. J. biol. Chem.* **264,** 8222–8231 (1989).
53. Wigler, M. *et al. Cell* **16,** 777–785 (1979).
54. Fabricant, R. F., DeLarco, J. E. & Todaro, G. J. *Proc. natn. Acad. Sci. U.S.A.* **74,** 565–569 (1977
55. Massague, J. *et al. J. biol. Chem.* **256,** 9419–9424 (1981).
56. Volonte, C. & Greene, L. A. *Growth Factors* **2,** 321–331 (1990).
57. Berg, M. M., Sternberg, D., Hempstead, B. L. & Chao, M. V. (manuscript submitted).
58. Yan, H., Schlessinger, J. & Chao, M. V. *Science* (in the press).
59. Hatakeyama, M. *et al. Science* **244,** 551–556 (1989).
60. Waldeman, T. A. *Ann. Rev. Biochem.* **58,** 875–911 (1990).
61. Sharon, M., Gnarra, J. R. & Leonard, W. J. *Proc. natn. Acad. Sci. U.S.A.* **87,** 4869–4873 (1990).
62. Burton, J. *et al. Proc. natn. Acad. Sci. U.S.A.* **87,** 7329–7333 (1990).
63. Hibi, M. *et al. Cell* **63,** 1149–1157 (1990).
64. Yayon, A., Klagsbrun, M., Esko, J. D., Leder, P. & Ornitz, D. M. *Cell* **64,** 841–848 (1991).
65. Morrison, D. K., Kaplan, D. R., Rhee, S. G. & Williams, L. T. *Molec. cell Biol.* **10,** 2359–2366 (1990
66. Kaplan, D. R. *et al. Cell* **61,** 125–133 (1990).
67. Margolis, B. *et al. Cell* **57,** 1101–1107 (1989).
68. Turner, J. M. *et al. Cell* **60,** 755–765 (1990).

69. Barde, Y.-A., Edgar, D. & Thoenen, H. *EMBO J.* **1,** 549–553 (1982).
70. Leibrock, J. *et al. Nature* **341,** 149–152 (1989).
71. Hohn, A., Leibrock, J., Bailey, K. & Barde, Y-A. *Nature* **334,** 339–341 (1990).
72. Maisonpierre, P. *et al. Science* **247,** 1446–1451 (1990).
73. Alderson, R. F., Alterman, A. L., Barde, Y.-A. & Lindsay, R. M. *Neuron* **5,** 297–306 (1990).
74. Rosenthal, A. *et al. Neuron* **4,** 767–773 (1990).
75. Ernfors, P. *et al. Proc. natn. Acad. Sci. U.S.A.* **87,** 5454–5458 (1990).
76. Lindsay, R. M., Thoenen, H. & Barde, Y.-A. *Devl Biol.* **112,** 319–328 (1985).
77. Ernfors, P., Wetmore, C., Olson, L. & Persson, H. *Neuron* **5,** 511–526 (1990).
78. Maisonpierre, P. *et al. Neuron* **5,** 501–509 (1990).
79. Rodriguez-Tebar, A. & Barde, Y.-A. *J. Neurosci.* **8,** 3337–3342 (1988).
80. Rodriguez-Tebar, A., Dechant, G. & Barde, Y.-A. *Neuron* **4,** 187–192 (1990).
81. Klein, R., Conway, D., Parada, L. & Barbacid, M. *Cell* **61,** 647–656 (1990).
82. Klein, R., Martin-Zanca, D., Barbacid, M. & Parada, L. F. *Development* **109,** 845–850 (1990).
83. Martin-Zanca, D., Barbacid, M. & Parada, L. F. *Genes Dev.* **4,** 683–694 (1990).

ACKNOWLEDGEMENTS. We thank J. Lown and M. Barkett for technical assistance, N. Patil for the pCMV5A construct, E. Johnson and J. Gorham for antibodies, M. Fuortes and L. Van Houton for assistance with the figures, J. Brugge for thoughtful discussion, and members of the Chao laboratory for advice. This research was supported by the National Cancer Institute and the DHHS; the March of Dimes, the Andrew W. Mellon Foundation and the National Cancer Institute (B.L.H.); from the NIH, ACS, the Hirschl/Caulier Trust Fund, Parke Davis and the Dorothy Rodbell Foundation (M.V.C).

LETTERS TO NATURE

Velocity structure of the ring nebula around supernova 1987A

Arlin P. S. Crotts* & Stephen R. Heathcote†

* Department of Astronomy, Columbia University, New York City, New York 10027, USA
† Cerro Tololo InterAmerican Observatory, Casilla 603, La Serena, Chile 1353

THE optical fading of supernova 1987A has revealed a parsec-sized nebula glowing in narrow atomic lines. The emission is presumed to come from circumstellar material, shed during the life of the progenitor star, illuminated by the ultraviolet flash from the explosion. This material is the closest to the supernova site that has been detected (apart from some transient radio emission immediately after the explosion[1]) and therefore represents the youngest pre-supernova structure available for study. Here we report a series of high-resolution spectra of the nebulosity. The hollow, 1.7-arcsec-wide nebula has the velocity field of a ring expanding at 10.3 km s^{-1}, not that of a limb-brightened spheroid. Fainter nebulosity within 3 arcsec is expanding slightly faster, as indicated by redshifted emission from behind the supernova. The geometry of the emission can be used to infer structure, provided that light travel time and recombination delays are accounted for. Our analysis sets an upper limit to the velocity of the wind from the progenitor during its red supergiant (RSG) phase, and implies an RSG lifetime of $<4\times10^5$ yr and an interval of $\sim2\times10^4$ yr between the end of the RSG phase and the supernova explosion.

Successive observations paint an increasingly detailed picture of the nebulosity. IUE detected circumstellar lines soon after the ultraviolet continuum decayed[2]. Their evolution indicated a size for the emission line region of $\sim2''$, optically confirmed by Wampler and Richichi[3], who reported a velocity gradient across the nebula, but not its direction or structural details. Imaging showed the nebula elongated east-west in optical lines (but invisible in the continuum), and centred in a fainter reflection echo and emission-line nebulosity[4]. Finer images showed the central nebula to be fainter in its core[5], probably a ring or limb-brightened spheroid. Hubble Space Telescope (HST) imaging showed this more clearly[12]. For the central nebula's velocity structure, Wood and Faulkner[6] reported a 2.5 km s^{-1} arcsec^{-1} gradient north-south and only 0.5 km s^{-1} arcsec^{-1} east-west. They did not describe the larger nebula, 5″ across. These and our results differ significantly from a third study[5].

We obtained spectra at resolution $R\approx30{,}000$ and in good seeing (often $<1''$ full width half maximum (FWHM)) with the $f/8$ echelle spectrograph on the 4-metre telescope at Cerro Tololo InterAmerican Observatory (CTIO), on days 741–742, 759, 909–910, 1024–1025, 1122–1124 and 1403–1405 after core collapse. The data have the supernova continuum removed by simple interpolation across the narrow emission lines. All velocities are heliocentric. The following line groups were observed simultaneously and at most epochs: Hα, [N II] lines at 6,548 Å and 6,584 Å, and [S4II] at 6,717 Å and 6,731 Å; Hβ and [O III] at 4,959 Å and 5,007 Å; [O II] at 3,726 Å and 3,729 Å and [Ne III] at 3,869 Å, and [N II] at 5,754 Å.

We first study the velocity field traced by the [N II] 6,584-Å line on day 741 (Fig. 1). Its centroid velocity is 289.2 km s^{-1}. We find little velocity shear across the central nebula at position angle PA = 60°, but large shears in opposing senses for position angles on either side, PA = 20° and 130°.

The morphology of the nebula arises from either a limb-brightened spheroid or an inclined and/or elongated ring. These have different implications for the nebula's kinematics. A circular ring with purely radial motion reproduces both the observed kinematics and morphology. To be consistent with HST imaging, such a ring would be inclined 43° from face-on with a semimajor axis, along PA = 100°, of 245 light-days (assuming the distance of the Large Magellanic Cloud $D_{\mathrm{LMC}} = 170{,}000$ light-years). An expansion velocity of 10.3 km s^{-1} reproduces the observed 14 km s^{-1} minor axis shear. The velocity field for this model (Fig. 1*d–f*), including seeing effects and spectrograph dispersion, matches the data at all position angles. The main deviations correspond to the larger echo loops discussed below.

This ring would be struck by the ultraviolet flash first on day 78, and last on day 409. These epochs correspond to when narrow ultraviolet lines 'turned on'[2], and later when many ultraviolet linestrengths began declining. This match, and the expansion velocity, would differ for an eccentric ring. This geometry also explains the lack of a reflection echo from the nebula on day 750 (ref. 4), despite more dimly fluorescent structures echoing strongly in the continuum. The nebula therefore extends less than ~300 light-days behind the supernova.

A sphere or prolate spheroid (with homologous velocity flow) generates no velocity shear on the projected minor axis, and oblate or triaxial solutions are highly constrained. To recover a shear of 14 km s^{-1} while limiting the velocity dispersion to <3 km s^{-1} as observed (with instrumental smearing deconvolved), an oblate or triaxial spheroid must be thinner than 1 : 5 in aspect ratio along PA = 10°. For such thin oblate spheroids, the minor axis inclination approaches 43°, resembling the proposed ring. No such constraint exists for the triaxial case. These results are less sensitive than light-curve constraints to holes in the nebula's matter distribution.

In the same spectra, Hα lines (Fig. 1*g–i*) show rough agreement with Fig. 1*d–f*, but there are details inconsistent with the model. Hα being broader than [N II] implies $T = 1.3\times10^4$ K. Electron temperatures predicted for portions of the nebula on day 742 are $1\text{-}4\times10^4$ K (ref. 7), albeit for a spherical shell model. Cooling rates have yet to be calculated for a ring and depend on the H I recombination time (S.R.H. and A.P.S.C., manuscript in preparation). The ring appears hotter to the south, farther from Earth, as expected from light travel delay.

Apart from the ring we see a spatially and spectrally compact spot at PA = 20° (feature 'A' in Fig. 1*g*), corresponding to the Hα source imaged on day 750 at PA = 10° (ref. 4). It vanished by day 909. Other spots are seen in images and spectra (also unresolved in velocity): feature 'B' in Fig. 1*i* (compare also ref. 8), and a fainter spot at PA = 165° in imaging on day 750 (ref. 4). All spots are just beyond the arclike echoes found within a

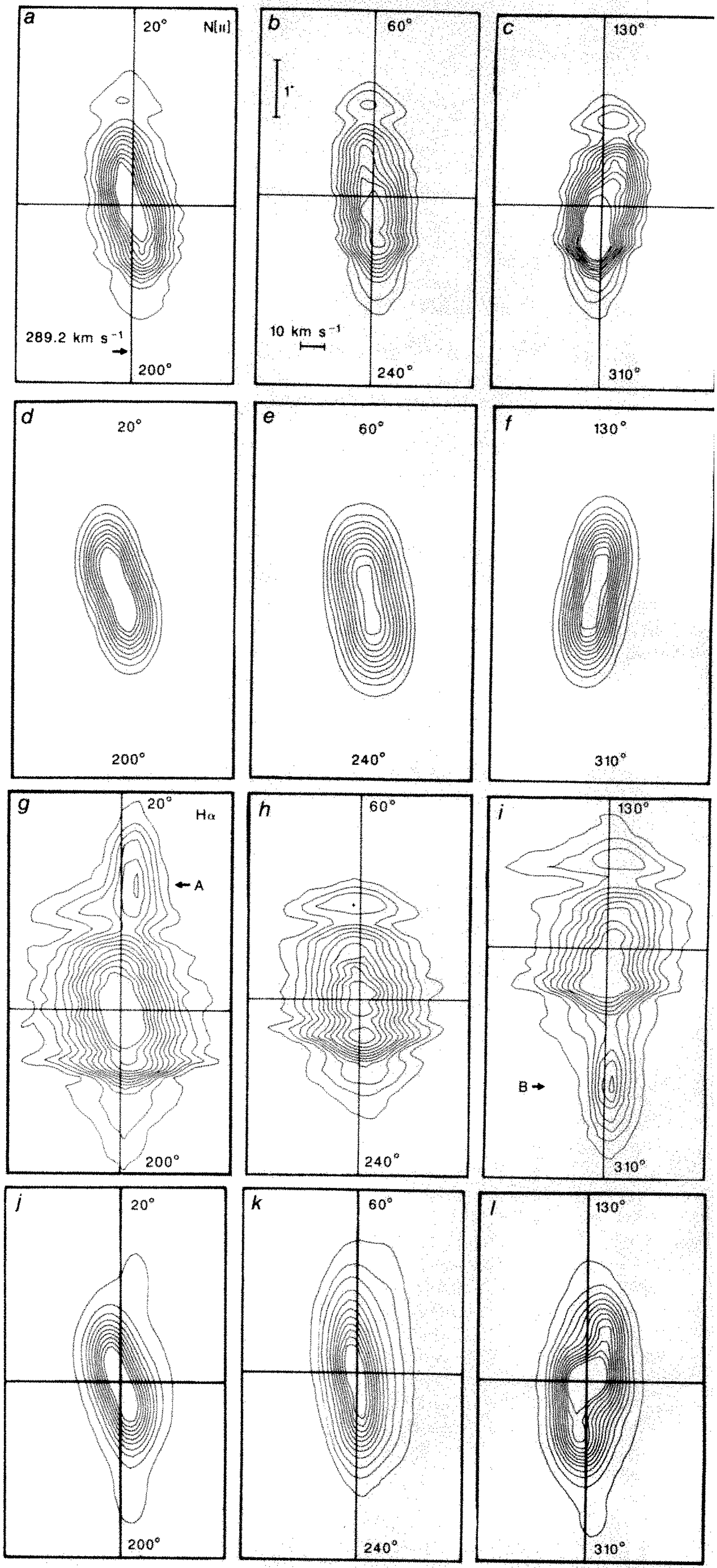


FIG. 1 Narrow [N II] emission at 6,584 Å around SN1987A on day 742 at different position angles of the spectrograph slit: *a*, 20°, *b*, 60° and *c*, 130°. The scale bars in *b* indicate units of slit length and velocity. Velocity increases to the right and the sense of the slit orientation is indicated by the arrow in *a*. The centroid velocity, 289.2 km s^{-1}, and the spatial position of the supernova are marked. *d–f*, As for *a–c*, but for the circular ring model described in the text. The same position angles are shown. *g–i*, The Hα line on day 742, for the same position angles. The features labelled A and B are described in the text. *j–l*, [N II] line at 6,584 Å on day 1402, for the same position angles.

radius $r = 3''$ and suggest instabilities along this interface between RSG and blue supergiant (BSG) winds. Elsewhere (S.R.H. and A.P.S.C., manuscript in preparation) we will use these spectra to estimate the properties of these clouds (as in ref. 4).

The faint, extended circumstellar features between these spots and the ring are important in understanding both. At radii $\sim 2''$ we see isolated line emission patches or extensions to the ring in both [N II] and Hα. These correspond to arcs first imaged on day 750 (ref. 4) that evolved only slightly in position by ~day 910 (refs 5, 9).

During our observations, the ultraviolet flash propagated from $2.6''$ from the supernova at right angles to the line-of-sight to $4.8''$ by day 1403. If these structures glow because of prompt recombination after the ultraviolet flash, they fall near this right-angle plane. If the velocities (measured from their lines) are due to radial outflow from the progenitor, their components along the line of sight should be small. Small offsets from the centroid velocity are indeed measured for the features at 1.5-$2.5''$. The tips of the ring in [N II] at PA $= 20°$, $130°$, $200°$ and $310°$ stand $\sim 7 \text{ km s}^{-1}$ to either side of the centroid, but the fainter extensions beyond $\sim 2''$ do not continue the gradient established by the ring.

These offsets are expected to evolve as the ultraviolet flash sweeps backwards into the nebulosity. If the $2.5''$ radius nebula is well approximated by a sphere[9] undergoing radial expansion, maximal change in redshift should have occurred during the interval of observation. Indeed, we see a shift of 7 km s^{-1} to the red during this time at PA $= 20°$ (Fig. 1*a* and *j*).

The brightest arc is the only echo at $r \ll 2.5''$ and therefore the only echoing material far behind the supernova. In Fig. 1*k*, we see this feature separating from the ring, redshifted 13 km s^{-1} from the centroid. This material expands away from the supernova. From Hα we find $T < 3{,}000$ K for this arc. The low temperature suggests that the velocity spread of the arcs at 1.5-$2.5''$ ($\sim 25 \text{ km s}^{-1}$ FWHM) is due to different bulk velocities smeared together by recombination delays, not a thermal effect (with $T \approx 20{,}000$ K). For instance, if the arcs expand at 15 km s^{-1}, then a 5×10^7 s recombination spread adds 12 km s^{-1} to the linewidth. This is supported by the subtle trend for red and blueshifted ends of the features in Hα and [N II] to bend inward, with the largest radius at the centroid velocity. This would occur if the nebula were concave towards the supernova as in a sphere. Also, the fact that some of the features at $r \approx 2.5''$ are asymmetrical in velocity argues against a thermal effect.

These data show mass expanding from the star, so the central ring is probably expanding, with its north side towards Earth. Matter also expands outside the ring plane, perhaps into bipolar lobes, the south lobe expanding into the supernova foreground. This locus of matter is inconsistent with that allowed above for oblate or triaxial spheroids. Furthermore, the 'zig-zag' shape of isophotes in data obtained with good seeing, for example Fig. 1*i*, is reproduced by the ring model but not by a model of a thin spheroid.

Even a crude understanding of the expansion of these structures constrains the mass-loss history of the progenitor. If the central ring is due to collision between the RSG and BSG winds, the expansion velocity of the gas in the ring, 10.3 km s^{-1}, is an upper limit to the RSG flow velocity. We can then establish the time between the RSG-to-BSG transition and the supernova explosion by comparing the expansion velocity with the ring radius. The time obtained is 2×10^4 years; the value depends on poorly known details of the acceleration of ring material by the BSG wind.

The RSG wind terminates in a second shock, 12 light-years in radius. Applying the argument of ref. 10 to the minimum velocity of 10.3 km s^{-1}, we derive a relatively firm lower limit to the duration of the RSG phase of 4.5×10^5 yr, with a mass loss rate of $2 \times 10^{-6} M_{\odot} \text{ yr}^{-1}$. There is about ten times as much mass accumulated in the terminus as there is still flowing outward, so the actual RSG lifetime could be much longer[9].

These time estimates agree with predictions from stellar evolution models[10,11]. The RSG minimum lifetime is consistent with predicted values such as 8×10^5 yr (ref. 10), but uncomfortably close if ten times as much mass was lost before $t_{\text{sn}} - 4.5 \times 10^5$ yr as afterwards. Stellar evolution models must satisfy both the limits on the RSG lifetime and the course of the mass-loss rate of the progenitor. ☐

Received 21 November 1990; accepted 6 March 1991.

1. Turtle, A. J. *et al. Nature* **327,** 38–40 (1987).
2. Fransson, C. *et al. Astrophys. J.* **336,** 429–441 (1989).
3. Wampler, E. J. & Richichi, A. *Astr. Astrophys.* **217,** L21–24 (1989).
4. Crotts, A. P. S., Kunkel, W. E. & McCarthy, P. J. *Astrophys. J.* **347,** L61–64 (1989).
5. Wampler, E. J., D'Odorico, S., Gouiffes, C., Tarenghi, M. & Wang, L.-F. *Astrophys. J.* **362,** L13–16 (1990).
6. Wood, P. R. & Faulkner, D. J. *IAU Circ.* No. 4739 (1989).
7. Lundqvist, P. & Fransson, C. *Astrophys. J.* (submitted).
8. Hanuschik, R. W. *Astr. Astrophys.* **237,** 12–16 (1991).
9. Crotts, A. P. S. & Kunkel, W. E. *Astrophys. J.* **366,** L73–77 (1990).
10. Woosley, S. E., Pinto, P. A. & Ensman, L. *Astrophys. J.* **324,** 466–489 (1988).
11. Saio, H., Kato, M. & Nomoto, K. *Astrophys. J.* **331,** 388–393 (1988).
12. Jakobsen *et al., Astrophys. J.* (in the press).

ACKNOWLEDGEMENTS. We thank E. Dwek and J. Felten for helpful discussions. This work began while A.P.S.C. was an NRC resident associate at Goddard SFC supported by NASA. Cerro Tololo InterAmerican Observatory, NOAO, is operated by Associated Universities for Research in Astronomy, Inc. under contract with NSF.

The physical state of the intergalactic medium

X. Barcons*, A. C. Fabian† & M. J. Rees†

* Departamento de Fisica Moderna, Universidad de Cantabria,
39005 Santander, Spain
† Institute of Astronomy, University of Cambridge, Madingley Road,
Cambridge CB3 0HA, UK

BECAUSE the process of galaxy formation is most unlikely to be perfectly efficient, there is a strong possibility that some baryonic gas remains outside collapsed structures such as galaxies and clusters of galaxies. What fraction of the baryonic content of the Universe resides in this intergalactic medium (IGM) and what physical state it is in are open questions. Here we use observational limits on the density of neutral hydrogen in the IGM, on the lack of deviations from a black-body spectrum of the cosmic microwave background (MBR), and on the extragalactic component of the soft X-ray background (XRB) to constrain the state of the IGM. From the lack of MBR fluctuations, any energetic IGM (containing as much energy as the binding energy in galaxies) is inferred to be smoothly distributed on scales greater than galactic. This rules out hot IGM models for the origin of the hard X-ray background, as well as the hypothesis that cosmic explosions may have given rise to cosmological structure on scales larger than galaxies.

When compared with the observed abundances of light elements, the standard theory of primordial nucleosynthesis (which assumes a perfectly homogeneous universe) places severe constraints on the fraction of the closure density of the Universe in the form of baryons[1]: $0.04 \leq \Omega_{\text{baryon}} \leq 0.1$. Here we assume $H_0 = 50 \text{ kms s}^{-1} \text{ Mpc}^{-1}$ and $q_0 = 0.5$ throughout. The upper limit on Ω_{baryon} can be somewhat relaxed with the use of models invoking density inhomogeneities during the nucleosynthesis era[2]. At redshift $z = 0$, luminous matter in galaxies already contributes $\Omega_{\text{baryon}} \approx 0.007$ (ref. 3); a similar amount is in intracluster gas[4], and some galactic or cluster baryonic dark matter could be present. A reasonable lower limit for the density of the IGM is $\Omega_{\text{IGM}} \geq 0.01$ at the present epoch.

There are, however, several recently updated observational facts that allow the density and temperature of the IGM to be constrained more directly, namely: (1) the lack of continuous absorption by neutral hydrogen (H I) shortward of the Lyman

α emission line in distant QSOs[5] (the Gunn–Peterson effect); (2) the upper limits to the extragalactic XRB at 0.25 keV (ref. 6) and 1 keV (ref. 7); (3) the lack of distortion in the submillimetre spectrum of the MBR[8]; and (4) improved limits on small-angular-scale anisotropy of the MBR. In a previous, complementary study[9], the properties of the ultraviolet ionizing flux were explored with the use of some of the above constraints. Here we use these constraints to gain information about the temperature of the IGM now and at an early epoch ($z = 4.5$).

The existence of significant amounts of diffuse H I in the IGM was ruled out many years ago[5] through meaurements of the Gunn–Peterson effect. Diffuse H I should produce an absorption trough shortward of the Lyman α emission line in the spectra of QSOs, but this has not yet been detected (constraint (1)). This means that the IGM must be highly ionized out to the highest reachable redshifts ($z \approx 5$).

The most stringent limit on the Gunn–Peterson effect has been obtained recently from a quasar at $z \approx 2.64$ by taking into account discrete absorption by Lyman α forest lines[10]. The inferred upper limit on the density is $n_{\rm H\,I} < 10^{-12}\ {\rm cm}^{-3}$ at tha redshift. Two main processes can contribute to the ionizatio of the IGM: photo-ionization by ultraviolet sources and collisions. The ultraviolet flux is not known with any accuracy At $z \approx 2$–3, QSOs produce a flux of only $J_0 \approx 10^{-21.7}\ {\rm erg\ cm^{-2}\ s^{-1}\ sr^{-1}\ Hz^{-1}}$ at the Lyman limit, but youn galaxies (if not obscured by dust) could increase this numbe by a factor of ~10 (ref. 11). The reflection model for the XRB[1 predicts a value for J_0 of $\sim 2\times10^{-21}\ {\rm erg\ cm^{-2}\ s^{-1}\ sr^{-1}\ Hz^{-1}}$ a the same redshift. Estimates of the ionizing flux at the presen epoch ($z = 0$) from H I boundaries of galaxies[13] suggest tha $J_0 \approx 4\times10^{-23}\ {\rm erg\ cm^{-2}\ s^{-1}\ sr^{-1}\ Hz^{-1}}$. This increases as $\sim(1+z)$ back to the redshift at which the bulk of the background i generated; but if the predominant sources of ultraviolet radiatio were at $z \gtrsim 3$ then J_0 could even rise more steeply than $\sim(1+z)$ beyond $z \approx 3$, where Lyman-limit absorption affects all lines o sight[11].

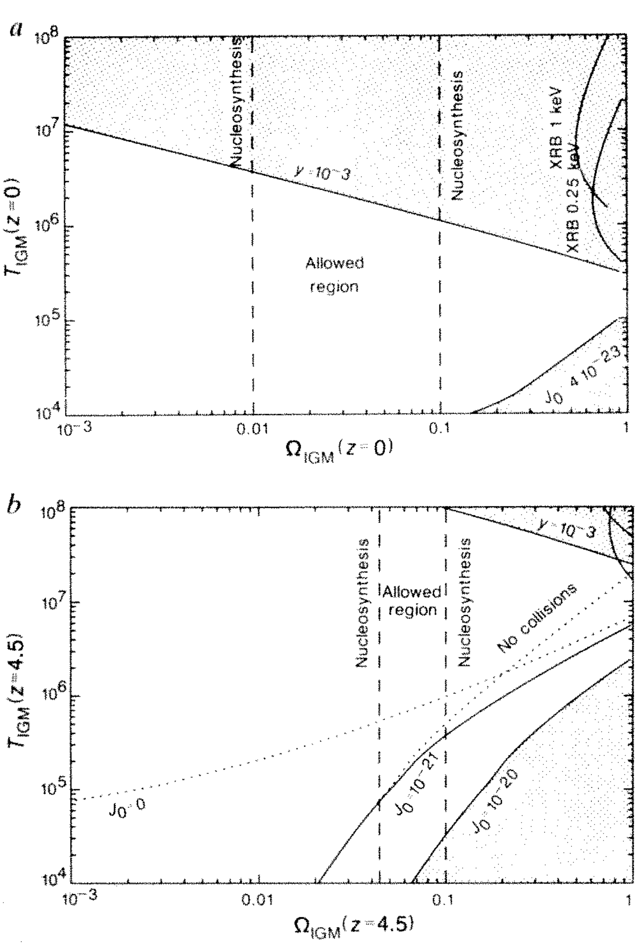


FIG. 1 *a*, Constraints on the temperature and density parameter for the IGM at the present epoch. The $y = 10^{-3}$ line represents the upper limit to $T_{\rm IGM}$ from COBE, and the curves labelled XRB denote the maximum IGM density from current upper limits to the extragalactic X-ray flux. Both assume adiabatic cooling from $z = 6$. The line marked $J_0 = 4\times10^{-23}$ is the minimum temperature as inferred from the absence of a Gunn–Peterson trough at $z = 2.64$ with an ultraviolet flux $J_0(z=0) = 4\times10^{-23}\ {\rm erg\ cm^{-2}\ s^{-1}\ sr^{-1}\ Hz^{-1}}$ evolving as $(1+z)^4$. The temperature of the IGM can be below the limit shown here only if there was a large energy input at the current epoch (so that the asumption of adiabatic cooling is incorrect). *b*, Same as *a* but at $z = 4.5$. The IGM is assumed to be suddenly reheated at $z = 4.5$ at the temperature shown. A Gunn–Peterson depression of less than 10% is assumed for several values of the ionizing flux at that redshift (in ${\rm erg\ cm^{-2}\ s^{-1}\ sr^{-1}\ Hz^{-1}}$). The minimum temperature is also shown for $J_0(z=4.5) = 10^{-21}\ {\rm erg\ cm^{-2}\ s^{-1}\ sr^{-1}\ Hz^{-1}}$ and no collisional heating.

In Fig. 1*a* we plot the minimum temperature required to kee the IGM ionized enough (that is, with $n_{\rm H\,I} \leqslant 10^{-12}\ {\rm cm}^{-3}$) a $z = 2.64$ as a result of collisions and photo-ionization, assumin a mean flux of $4\times10^{-23}\ {\rm erg\ cm^{-2}\ s^{-1}\ sr^{-1}\ Hz^{-1}}$ at $z = 0$ whic evolves as $(1+z)^4$. Ionization equilibrium is assumed[14]. Th heating and cooling processes that we consider for determinatio of the $z = 0$ temperature are adiabatic cooling, ultraviolet heating, collisional ionization and recombination cooling. As wa discussed in ref. 9, this photo-ionization rate is sufficient t satisfy the Gunn–Peterson constraint unless $\Omega_{\rm IGM} \gtrsim 0.1$. I photo-ionization is still important at the present epoch, th temperature probably does not drop significantly below 10^4 K Analogous limits on the IGM temperature are shown in Fig. 1 for $z = 4.5$. We assume that the Gunn–Peterson trough there ha a depth of less than 10%. The level of the ionizing backgroun is highly uncertain at this very high redshift: there may be fewe photo-ionizing sources, but on the other hand absorption b intervening Lyman-limit clouds may mean that extrapolation of lower redshift estimates of J_0 are underestimated. If ther were no heating input other than photo-ionization, $T_{\rm IG}$ could not exceed 2×10^4 K. Figure 1*b* therefore shows tha with a modest ionizing ultraviolet flux ($J_0 \approx 10^{-21}\ {\rm erg\ cm^{-2}\ s^{-1}\ sr^{-1}\ Hz^{-1}}$) $\Omega_{\rm IGM}$ cannot exceed ~0.03 unles there is some collisional heating. This figure also shows explicitl how hot the gas must be for different values of J_0. If J_0 wer so small that photo-ionization were negligible, the temperatur of the IGM at $z \sim 4.5$ would have to be at least a few time 10^5 K for $\Omega_{\rm IGM}$ in the range 0.01–0.03. The lower limit to T rise roughly proportional to $\Omega_{\rm IGM}$ for higher densities.

The most recent upper limits[6,7] to the extragalactic soft X-ra intensity (constraint (2) above) are $I_{\rm X}(0.25\ {\rm keV}) \lesssim 60\ {\rm keV\ cm^{-2}\ s^{-1}\ keV^{-1}}$ and $I_{\rm X}(1\ {\rm keV}) \leqslant 20\ {\rm keV\ cm^{-2}\ s^{-1}\ keV^{-}}$ The contribution of the IGM to the soft X-ray backgroun depends on its clumpiness, characterized by the clumping fact

$$C = \left(\frac{\langle n_{\rm IGM}^2 \rangle - \langle n_{\rm IGM} \rangle^2}{\langle n_{\rm IGM} \rangle^2} \right)^{1/2}$$

The bremsstrahlung volume emissivity is proportional to $n_{\rm IGM}^2$ so $I_{\rm X} \propto (1 + C^2)$. The temperature-dependent upper limits t $\Omega_{\rm IGM}$ for a uniform IGM ($C = 0$) are shown in Fig. 1, wher the intensities have been calculated for a constant gaunt factor[1 which provides a good approximation for our purposes. reheating redshift $z_{\max} = 6$ (just enough to fulfill the Gunn Peterson effect) and subsequent adiabatic cooling have bee assumed for Fig. 1*a*.

Deviations from a purely planckian spectrum in the submill metre region of the MBR are usually parameterized in terms the comptonization y-parameter, which is defined as the averag shift in the logarithm of the frequency for a microwave photo The first results from the Cosmic Background Explorer (COBE give an upper limit of $y < 10^{-3}$ (constraint (3)). The Compto distortion parameter, y, measures the integrated pressure alon

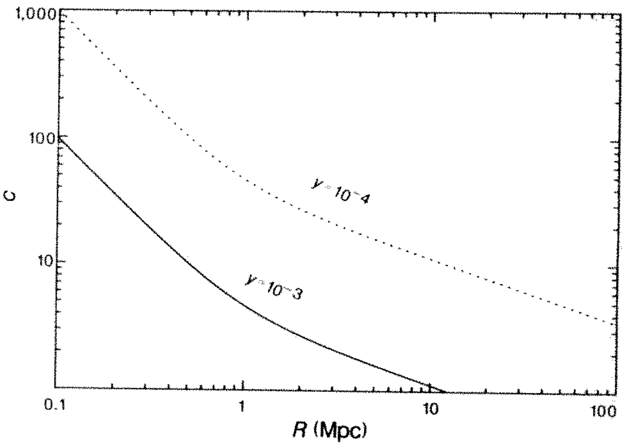


FIG. 2 Maximum allowed values of the clumping factor C as a function of comoving clumping radius, R, for several values of the Compton distortion parameter, from $(\Delta T/T)_{\mathrm{MBR}} < 2\times10^{-5}$ for a beamwidth of 1 arcmin (ref. 19).

the line of sight in the IGM, increased by a factor of $(1+4kT/m_ec^2)$, which is important at high temperatures. (We again assume simple adiabatic cooling for the gas, which allows us to place the upper limits shown in Fig. 1.)

These three observational constraints produce a picture of a uniform IGM consisting of a highly ionized gas with a temperature (at $z=0$) of $10^4\,\mathrm{K} \leq T_{\mathrm{IGM}} \leq 10^7\,\mathrm{K}$. As a first conclusion, the hard X-ray background[16] at 3–100 keV cannot arise as thermal bremsstrahlung in a hot uniform IGM, as that would require $T \gtrsim 10^8$ K and $\Omega_{\mathrm{IGM}} \approx 0.25$ (ref. 17), conflicting strongly with the COBE results.

At the more likely temperatures of $\sim 10^4$ K, however, there are reasons to expect the IGM to be inhomogeneous. Strong localized sources (such as QSOs) will heat up their surroundings above the average, and deep, extended potenial wells (such as clusters of galaxies) will concentrate high-density gas at temperatures of $\sim 10^7$ K. Therefore, we must explore the possibility that the IGM is significantly clumpy.

To do this, we assume a simple model in which randomly distributed spherical blobs with gaussian gas-density profiles contain all of the IGM gas[18]. The evolution of the clumping factor C is uncertain, but is most important at the highest redshifts where most of the background is generated. We assume that C is independent of redshift.

An obvious consequence of clumping is that the soft X-ray background curves in Fig. 1 have to be shifted towards lower values of Ω_{IGM} by a factor of order $1/C$. Therefore, if $C \gtrsim 10$ these constraints become relevant, and for $C \approx 100$ they require $\Omega_{\mathrm{IGM}} \leq 0.01$. Furthermore, the soft X-ray background would also show important fluctuations on the clumping scale.

But a much stronger constraint than the isotropy of the soft X-ray background comes from the lack of angular fluctuations in the MBR (constraint (4)). If $C>0$, the MBR will show fluctuations owing to the fact that microwave photons will be scattered by a finite number of IGM blobs along the line of sight. To our knowledge, the most stringent limit is $(\Delta T/T)_{\mathrm{MBR}} \leq 2\times10^{-5}$ for a beamwidth of ~ 1 arcmin (ref. 19). The expected level of fluctuations in the MBR for a clumpy IGM is of the order of $(\Delta T/T)_{\mathrm{MBR}} \sim y/\sqrt{N_{\mathrm{b}}}$, where y is the total Compton distortion parameter and N_{b} is the total number of IGM blobs per beam. Figure 2 shows the maximum clumping factor C on different scales coming from the above upper limit on $(\Delta T/T)_{\mathrm{MBR}}$, where beamwidth effects have been properly included. The result is that significant clumping ($C \gtrsim 10$) can occur only on scales $\lesssim (y/10^{-3})^{-1}$ Mpc.

This has a number of implications for specific models. First, there is the possibility already mentioned that the 3–100-keV X-ray background could arise as thermal bremsstrahlung in the IGM ($T_{\mathrm{IGM}}(z=0) \gtrsim 10^8$ K). Even if the reheating redshift is as low as ~ 4, the maximum amount of IGM at this temperature allowed by the COBE upper limit ($y<10^{-3}$) is $\Omega_{\mathrm{IGM}} \approx 0.01$, whereas $\Omega_{\mathrm{IGM}} \approx 0.25$ is required for a uniform IGM that gives rise to the hard XRB[17,18]. This results in a minimum clumping factor $C \gtrsim 25$, and if upper limits on y decrease significantly, bigger clumping factors would be required. According to Fig. 2, this means that the hot gas should be confined to blobs with a size below ~ 0.5 Mpc, which corresponds to the scale of a galaxy. No class of point-like source has yet been found that has a spectrum similar to the 3–100-keV X-ray background. Clearly, if the X-ray background arises from such a source, it is not from the IGM: the XRB must come from point sources.

A second important consequence concerns the explosion scenario for galaxy formation[20]. In this picture, galaxies explode and throw an energy equivalent to their binding energy into the IGM. When the bubbles finally overlap, they must have typical sizes of several Mpc, because the model aims to reproduce the structure of the Universe up to ~ 10 Mpc. Now, this model produces $y \approx 10^{-3}$ with very significant clumping on scales much greater than ~ 1 Mpc, which is not allowed by the isotropy of the MBR.

A further possibility that has to be considered is that the metallicity of the IGM might be similar to that of intracluster gas (that is, $\sim 0.3Z_{\odot}$, where $Z_{\odot}$ is the solar metal/hydrogen density ratio). This is expected if the ratio of galaxy to gas density in the Universe is constant. The possibility of detecting resonance absorption lines from highly ionized species (such as O VIII) in the IGM towards distant featureless X-ray sources (such as BL Lacs[21]) is then an important and observable diagnostic for the composition of the IGM. □

Received 8 November 1990; accepted 28 January 1991.

1. Boesgaard, A. M. & Steingman, G. *A. Rev. Astr. Astrophys.* **23,** 319–378 (1985).
2. Malaney, R. A. & Fowler, W. A. *Astrophys. J.* **333,** 14–20 (1988).
3. Faber, S. M. & Gallagher, J. S. *A. Rev. Astr. Astrophys.* **17,** 135–187 (1979).
4. Sarazin, C. L. *Rev. mod. Phys.* **58,** 1–115 (1986).
5. Gunn, J. E. & Peterson, B. A. *Astrophys. J.* **142,** 1633–1636 (1965).
6. MacCammon, D. & Sanders, W. T. *A. Rev. Astr. Astrophys.* (in the press).
7. Wu, X., Hamilton, T., Helfand, D. J. & Wang, Q. *Astrophys. J.* (in the press).
8. Mather, J. C. *et al. Astrophys. J.* **354,** L37–L40 (1990).
9. Miralda-Escudé, J. & Ostriker, J. P. *Astrophys. J.* **350,** 1–22 (1990).
10. Steidel, S. & Sargent, W. L. W. *Astrophys. J.* **318,** L11–L14 (1987).
11. Bechtold, J., Weymann, R. J., Lin, Z. & Malkan, M. A. *Astrophys. J.* **315,** 180–197 (1987).
12. Fabian, A. C., George, I. M., Miyoshi, S. & Rees, M. J. *Mon. Not. R. astr. Soc.* **242,** 14P–16P (1990).
13. Kenney, J. D. P. in *The Interstellar Medium in Galaxies* (eds Thronson, H. A. & Shull, J. M.) 151–180 (Kluwer, Dordrecht, 1990).
14. Black, J. H. *Mon. Not. R. astr. Soc.* **197,** 553–563 (1981).
15. Sherman, R. D. *Astrophys. J.* **256,** 370–373 (1982).
16. Marshall, F. E. *et al. Astrophys. J.* **235,** 4–10 (1980).
17. Guilbert, P. W. & Fabian, A. C. *Mon. Not. R. astr. Soc.* **220,** 439–451 (1986).
18. Barcons, X. & Fabian, A. C. *Mon. Not. R. astr. Soc.* **230,** 189–206 (1988).
19. Partridge, R. B. in *Physical Cosmology* (ed. Trân Thanh Vân, J. (Editions Frontières, Gif-sur-Yvette, in the press).
20. Ostriker, J. P. & Cowie, L. L. *Astrophys. J.* **243,** L127–L132 (1981).
21. Canizares, C. R. & Kruper, J. *Astrohys. J.* **278,** L99–L102 (1984).

A nearby 37.9-ms radio pulsar in a relativistic binary system

A. Wolszczan

Arecibo Observatory, Arecibo, Puerto Rico 00613, USA

WITH very few exceptions[1], high Galactic latitudes have not been surveyed with sufficient sensitivity to detect millisecond pulsars. Accordingly we have conducted a preliminary sensitive survey at Galactic latitudes $b \geq 30°$ using the Arecibo 305-m radiotelescope at a frequency of 430 MHz. Here I report the discovery of a 37.9-ms radio pulsar, PSR1534+12, in a 10.1-hour eccentric binary orbit[2]. Timing analysis constrains the masses of the pulsar and its companion to be $1.32 \pm 0.03\ M_{\odot}$ and $1.36 \pm 0.03\ M_{\odot}$ (where $M_{\odot}$ is the mass of the Sun). This, together with the high eccentricity ($e = 0.274$) and small orbital diameter ($\sim 2\ R_{\odot}$), indicates that the companion is probably another neutron star, and that the orbital evolution is strongly influenced by effects due to general relativity. The exceptionally high timing accuracy obtainable in PSR1534+12, because the pulse is so strong and narrow, will allow general relativity to be tested with unprecedented accuracy. The unique morphology of the pulsar's emission and polarization will make possible the measurement of a previously unconfirmed relativistic effect, the geodetic precession of the pulsar spin-axis.

The Arecibo survey was carried out in February 1990 during an extensive maintenance period, when the restrictions on the telescope motion limited its usage to transit observations. The ranges of Galactic longitudes $0° \leq l \leq 35°$ and $210° \leq l \leq 360°$ were drift-scanned for 4-5 h per day at constant elevation. This provided an integration time of 34 s within the half-power limits of the 10′ telescope beam. The data were acquired in the manner described in earlier reports[3]. With the sampling interval of 506.625 μs and the above integration time, this scheme generated 65,536 data samples per beam in each of the 128 frequency channels over a 10-MHz receiver bandwidth and provided a 6σ sensitivity of ~1.5 mJy down to pulsar periods of ~4 ms. In three weeks of observations, the survey covered an area of the sky ~150 square degrees. The data were analysed at the Cornell National Supercomputer Facility using the standard pulsar search procedure[3].

The 37.9-ms pulsar (Fig. 1) was first detected in the data obtained on 4 February 1990. In addition, an analysis of the observations made on 9 February revealed the presence of another pulsar, PSR1257+12, with a period of 6.2 ms. Follow-up timing measurements indicate that PSR1257+12 is probably member of a long-period binary system. Here I will concentrat on the description of the PSR1534+12 system, postponing th discussion of PSR1257+12 until its parameters are known mor accurately.

TABLE 1 Parameters of the PSR1534+12 binary system

Pulsar period, P	$0.0379044403665 \pm 0.0000000000004$ s
Period derivative, $\dot{P}$	$2.43 \times 10^{-18} \pm 8 \times 10^{-20}$ s s^{-1}
Right Ascension (B1950.0)	15 h 34 m 47.686s ± 0.003
Declination (B1950.0)	12° 05′ 45.23″ ± 0.03
Epoch	JD 2448200.0
Dispersion measure	11.61 ± 0.01 pc cm^{-3}
Flux density (430 MHz)	36 ± 8 mJy
Projected semi-major axis, $a_1 \sin i$	3.72943 ± 0.00003 light-s
Eccentricity, e	0.2736761 ± 0.0000007
Epoch of periastron, T_0	JD $2448199.8121643 \pm 0.0000003$
Orbital period, P_b	36351.7027 ± 0.0003 s
Longitude of periastron, ω	$264.672 \pm 0.0006°$
Periastron advance, $\dot{\omega}$	$1.7562 \pm 0.0015°$ yr^{-1}
Time dilation, gravitational redshift, γ	0.0017 ± 0.0004 s
Orbital inclination, i	~74°
General relativity model masses and derived parameters	
Total mass, M	$2.679 \pm 0.003\ M_{\odot}$
Companion mass, m_2	$1.36 \pm 0.03\ M_{\odot}$
Pulsar mass, $m_1 = M - m_2$	$1.32 \pm 0.03\ M_{\odot}$
Mass function $f(m_1, m_2)$	$0.3146 \pm 0.0002\ M_{\odot}$
Pulsar surface magnetic field, B	1.0×10^{10} G
Pulsar characteristic age, τ_c	2.4×10^{8} yr

The shape and amplitude of the measured radial velocit curve shown in Fig. 2 indicate that PSR1534+12 is in an eccen tric orbit around a massive companion. I obtained a phase connected solution for the rotational and positional parameter of the pulsar, the keplerian orbit and the first two post-kepleria parameters $\dot{\omega}$ (rate of periastron advance) and γ (variable pa of time dilation and gravitational redshift) from a least square fit of the relativistic binary timing model of Damour an Deruelle[4,5] to the observed arrival times of the pulses, using th analysis program TEMPO[5] and the Center for Astrophysic Solar System ephemeris PEP740R. Pulsar parameters derive from the fit to the data spanning the period from February t December 1990 are listed in Table 1. The residuals after fittin of observations made with the bank of 32 contiguous 250-kH filters and the Princeton Mark III pulsar processor at 430 MH did not exceed 5 μs for integration times of 2.5 minutes. Pr liminary analysis indicates that reduction of this residual to 2 μ or less is possible.

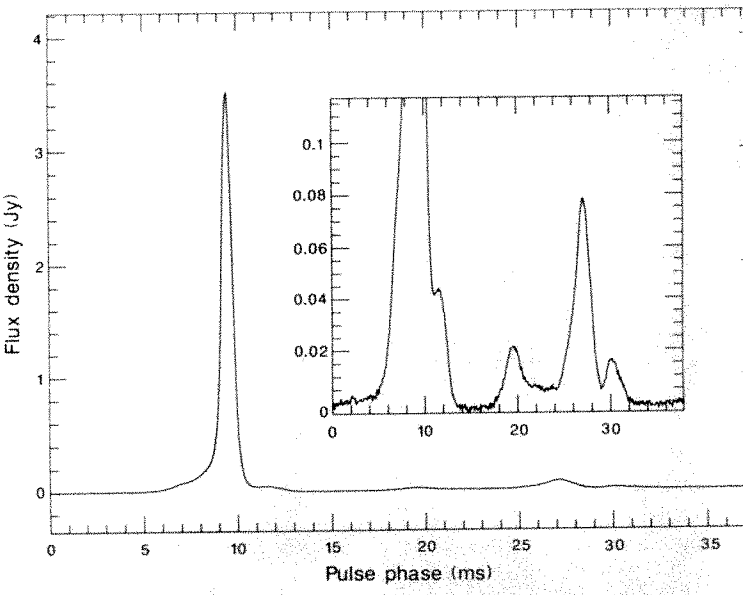


FIG. 1 The average pulse profile of PSR1534+12 at 430 MHz. The effective time resolution is ~300 μs. The inset shows low-level emission enhanced by a factor of 20. The main pulse width at half-maximum is ~450 μs.

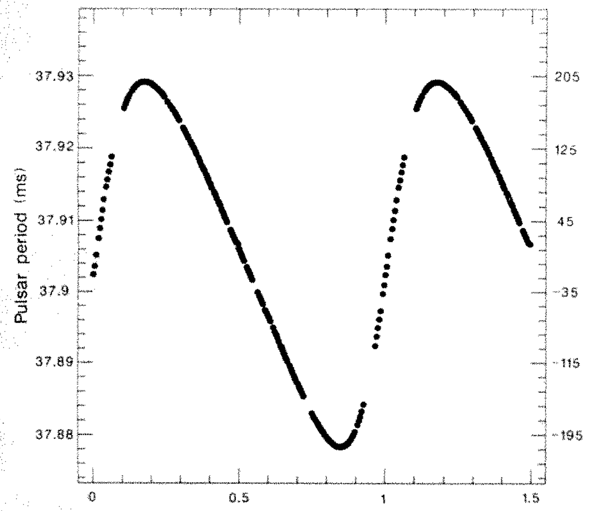


FIG. 2 The radial velocity curve of the orbital motion of PSR1534+12.

PSR1534+12 is evidently a member of a binary system whose parameters are similar to those of the canonical high-mass binary pulsar 1913+16 (ref. 5). From the facts that the minimum separation between the companion stars is ~1 $R_\odot$, that no eclipses are observed in the system and that the pulsar timing residuals show no significant deviations from the assumed point-mass binary model, it is almost certain[6] that the companion of PSR1534+12 is another compact object. Moreover, the small slowdown rate of the pulsar implies that it is old and has a weak magnetic field (Table 1). These characteristics are typical of pulsars spun up by accretion of matter from binary companions and suggest that 1534+12 is the end-product of the evolution of a massive binary system[7].

Masses of the two stars can be derived using an explicitly general-relativistic approach[5] in which the post-keplerian orbital parameters are expressed in terms of a total system mass M and a companion mass m_2. A fit of this model to the 1534+12 data gives accurate values of these parameters, $M = 2.679 \pm 0.003\ M_\odot$ and $m_2 = 1.36 \pm 0.03\ M_\odot$, immediately leading to a pulsar mass $m_1 = M - m_2 = 1.32 \pm 0.03\ M_\odot$. These quantities are included in Table 1, and constraints imposed on the masses by the measured post-keplerian timing parameters in this model, and in the model in which $\dot{\omega}$ and γ are free parameters[4,5], are illustrated in Fig. 3.

The masses of PSR1534+12 and its companion are within the range of neutron star masses determined from observations of other radio and X-ray pulsars[5,8-11]. They also agree with masses estimated from theoretical considerations of neutron star structure[12] and the mechanism of supernova explosions[13]. The component masses of the 1534+12 system agree well with the theoretically predicted 1.3-1.35 $M_\odot$ for the gravitational mass of a neutron star[13]. The masses in the 1913+16 system, 1.442 $M_\odot$ and 1.386 $M_\odot$ (ref. 5), and the 1534+12 data presented here are the most accurate determinations of neutron star mass so far.

As discussed above, the companion of PSR1534+12, apparently the heavier of the two objects, is very likely to be another neutron star. According to the standard model[7], it was formed as the result of a collapse of its helium star progenitor. Assuming a spherical explosion, a straightforward calculation[14] using the observed eccentricity and the pulsar mass leads to a progenitor mass of ~2.1 $M_\odot$. This is at the theoretical 'soft' lower limit to a helium core mass that would still collapse to a neutron star (2.2-2.5 $M_\odot$, refs 13, 15).

The observed eccentricity, as well as other orbital parameters, could be affected by an asymmetry in the supernova explosion of a more massive helium star[14,16,17]. Preliminary modelling of the geometry of PSR1534+12 (A.W. and J. A. Phillips, manuscript in preparation) using the observed polarization of the extended off-pulse emission (Fig. 1) indicates that the spin axis of the pulsar is inclined to the direction of the orbital angular momentum by 17° ± 2° given the orbital inclination in Table 1. This result strongly suggests that the supernova explosion that gave rise to the pulsar's companion was indeed asymmetric.

In principle, the observed eccentricity could also result from a significant circularization of the orbit caused by gravitational radiation[5,6] during orbit decay. Given the orbital parameters of PSR1534+12 and the masses of the companion stars, the theoretical rate of orbital decay ($\dot{P}_b \approx -1.9 \times 10^{-13}$) leads to a time for orbital dissipation ($\sim P_b/\dot{P}_b$) in excess of 10^9 yr. As this is much longer than the characteristic age of the pulsar ($\sim 2 \times 10^8$ yr), it is unlikely that the eccentricity observed now differs much from its value just after formation of the second neutron star.

PSR1534+12 exhibits a very rare combination of properties which make it a unique astrophysical object. Most notably, the pulsar's membership of a relativistic binary system offers new, fascinating opportunities to study gravity under strong-field conditions. The possibility of achieving timing accuracy of better than 2 μs for PSR1534+12, as compared with ~20 μs for PSR1913+16 (ref. 5) and ~65 μs for PSR2127+11C (ref. 9), means that relativity theories can be tested with unprecedented precision. Furthermore, the presence of a significant misalignment of the pulsar spin with the orbital angular momentum in the 1534+12 system means that the relativistic geodetic precession of the pulsar spin axis[6] at the rate of ~0.5° yr^{-1} should manifest itself in the form of changes in the pulse shape, amplitude and polarization, as the pulsar beam drifts past the observer's line of sight. Results suggesting that this does occur in the 1913+16 system[18,19] have not been conclusive in the absence of a constraint provided by the measurement of a spin-orbit angle. As this angle has already been measured for PSR1534+12, further long-term monitoring of its pulse arrival times, morphology and polarization should allow the existence of geodetic precession to be confirmed unambiguously.

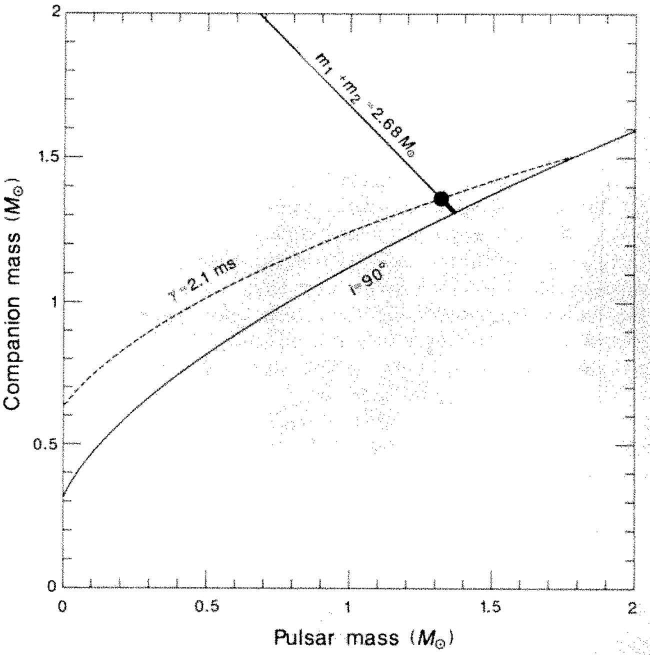


FIG. 3 General-relativistic constraints on masses of PSR1534+12 and its companion. A permissible range of masses (the thickened part of the $m_1 + m_2$ line) is delimited by the total mass derived from $\dot{\omega}$, the upper limit to γ and the $i = 90°$ (edge-on orbit) condition imposed by the observed mass function. The masses obtained from the explicitly general-relativistic model are indicated by the filled circle, whose size reflects the estimation uncertainties.

Detections of PSR1534+12 and the 6.2-ms pulsar PSR1257+12 reported in this paper are important for our understanding of millisecond pulsar statistics[20,21]. Both pulsars are at high Galactic latitudes, have low intrinsic luminosities (8–10 mJy kpc^2 at 400 MHz) and are at distances ≤0.5 kpc. Their discovery provides new evidence for the existence of a large population of old, 'spun up' pulsars in the Galaxy and indicates that future all-sky surveys have a good chance of detecting more millisecond pulsars in the solar neighbourhood. ☐

Received 7 January; accepted 13 March 1991.

1. Stokes, G. H. *et al. Astrophys. J.* **311**, 694–700 (1986).
2. Wolszczan, A. *IAU Circular No.* 5073 (1990).
3. Wolszczan, A. *et al. Nature* **337**, 531–533 (1989).
4. Damour, T. & Deruelle, N. *Ann. Inst. H. Poincaré (Physique Théoretique)* **44**, 263 (1986).
5. Taylor, J. H. & Weisberg, J. M. *Astrophys. J.* **345**, 434–450 (1989).
6. Smarr, L. L. & Blandford, R. *Astrophys. J.* **207**, 574–588 (1976).
7. van den Heuvel, E. P. J. & Taam, R. E. *Nature* **309**, 235–237 (1984).
8. Nagase, F. *Publs astron. Soc. Japan* **41**, 1–79 (1989).
9. Anderson, S. B., Gorham, P. W., Kulkarni, S. R., Prince, T. A. & Wolszczan, A. *Nature* **346**, 42– (1990).
10. Lyne, A. G. & Bailes, M. *Mon. Not. R. astr. Soc.* **246**, 15P–17P (1990).
11. Ryba, M. F. & Taylor, J. H. *Astrophys. J.* **371**, in press.
12. Baym, G. & Pethick, C. J. *Ann. Rev. astr. Astrophys.* **17**, 415–443 (1979).
13. Woosley, S. E. in *The Origin and Evolution of Neutron Stars, IAU Symp. 125* (eds Helfand, D. & Huang, J. H.) 255–272 (Reidel, Dordrecht, 1986).
14. Flannery, B. P. & van den Heuvel, E. P. J. *Astr. Astrophys.* **39**, 61–67 (1975).
15. Habets, G. M. H. J. *Astr. Astrophys.* **167**, 61–76 (1986).
16. Hills, J. G. *Astrophys. J.* **267**, 322–333 (1983).
17. Burrows, A. & Woosley, S. E. *Astrophys. J.* **308**, 680–684 (1986).
18. Weisberg, J. M., Romani, R. W. & Taylor, J. H. *Astrophys. J.* **347**, 1030–1033 (1989).
19. Cordes, J. M., Wasserman, I. & Blaskiewicz, M. *Astrophys. J.* **349**, 546–552 (1990).
20. Bhattacharya, D. & Srinivasan, G. *Curr. Sci.* **55**, 327–330 (1986).
21. Kulkarni, S. R. & Narayan, R. *Astrophys. J.* **335**, 755–769 (1988).

ACKNOWLEDGEMENTS. I thank J. H. Taylor for helpful comments and for making available the Ma III equipment and the latest version of TEMPO; also M. F. Ryba for assistance with the Mark observing. Arecibo Observatory is part of the National Astronomy and Ionosphere Center, which operated by Cornell University under contract with the NSF. Computations were performed at t Cornell National Supercomputer Facility which is supported in part by NSF, IBM Corporation, New Yo State and the Cornell Research Institute.

Anomalous neutron scattering near the superconducting phase transition

N. R. Bernhoeft*, P. J. Allen*, D. McK. Paul†, S. M. Hayden‡, P. A. Timmins‡ & G. G. Lonzarich§

* Physics Department, University of Durham, South Road,
Durham DH1 3LE, UK
† Physics Department, University of Warwick, Coventry CV4 7AL, UK
‡ Institut Laue-Langevin, 156X, 38042 Grenoble Cedex, France
§ Interdisciplinary Centre on Superconductivity and
Cavendish Laboratory, University of Cambridge,
Madingley Road, Cambridge CB3 0HE, UK

THE inelastic scattering of low-energy neutrons near a second-order phase transition can provide a description of the spontaneous fluctuations in space and time of the physical parameter which, at low temperatures, enters a state of long-range order. Often the order parameter is coupled to the neutron directly, as in magnetic or lattice relaxation phenomena; as in the case of superconductivity, discussed here, the coupling is indirect. In either case, inelastic neutron diffraction measures the amplitude of the equilibrium fluctuations on an absolute scale, which may be compared with the predictions of models based on linear response theory[1]. To this end, we report an investigation of the temperature dependence of small-angle neutron scattering in homogeneous bulk samples of the high-temperature superconductor $YBa_2Cu_3O_{7-\delta}$. An anomalous increase in the scattering intensity occurs near the superconducting phase transition, and at lower temperatures. These effects seem to be larger than can be accounted for in elementary models of the normal and superconducting states.

The order parameter associated with the superconducting state may couple to the neutron in several indirect ways. Fluctuations in the amplitude and of the gradient of the phase of the order parameter generate slow fluctuations in the electron currents which couple to the neutron dipole moment. In a semi-classical picture, the currents act as sources of position- and time-dependent magnetic fields which diffract the neutron wave. If, on the appropriate space and time scales, the order parameter is also accompanied by fluctuations in the local spin density, this too may be seen as a source of fluctuating magnetic field[2]. In addition, there are secondary sources of scattering in which the primary fluctuations of the order parameter modulate available degrees of freedom to which the neutron is directly sensitive. Thus, for example, longitudinal (charge-density) modulations of the superconducting order parameter may drive low-frequency ionic density oscillations which will be seen through the nuclear scattering potential, and modulations in the order parameter will give rise to scattering from fluctuation induced in the normal electron fluid itself.

Superconducting materials with elevated transition temperatures offer a unique opportunity to gain insight into th dynamical properties of the superconducting order paramete by inelastic neutron scattering techniques. This arises for tw reasons: first, the elevated temperatures allow a significant thermal population to be put into low-energy excitations; second the contracted spatial scale of pairing, more or less implied b the high transition temperature (T_c) and the inferred magnitud of the effective binding energy allows measurements to be mad at experimentally accessible scattering angles. We can thu explore fluctuations of low frequencies, $\omega \ll kT_c/\hbar$, and o characteristic wavelengths λ in the range $\xi_c < \lambda < \lambda_L$, where is the superconducting pair coherence length and λ_L is th London screening length, without losing the response becaus of dynamical screening of magnetic fields (for wavelength greater than λ_L) or the characteristic stiffening of the respons (for wavelengths shorter than ξ_c).

Samples of $YBa_2Cu_3O_{7-\delta}$ were prepared as small, polycrysta line, sintered discs from materials of the highest quality, takin great care to maximize homogeneity at every stage of manufacture. Magnetization, resistive and X-ray studies confirmed the purity, phase composition and superconducting nature at temperatures below 91 ± 1 K.

The temperature dependence of small-angle scattering cross section from the high-purity $YBa_2Cu_3O_{7-\delta}$ samples is shown i Fig. 1. The data show a clear increase in intensity over th background level as the material passes through the superconducting transition. This scattering is broadly distributed abou the transition temperature over a range of ~10 K, which is mor than an order of magnitude wider than the bulk screenin transition region measured either inductively or resistively. Th intensity of the excess scattering near T_c decreases with increasing wavevector. Below the phase transition a broad anomal with a peak at ~30 K is seen. This too decreases in intensit with increasing wavevector.

The interpretation of the data fall naturally into two parts first, that at elevated temperatures where the increased scatterin signals the build-up of short-range order and the transitio to the condensed state, and second, the scattering observe in the state of long-range order below T_c. We focus primaril on the changes taking place close to or just above the transition temperature, where long-wavelength and low-frequenc fluctuations of the order parameter are normally mo pronounced.

For reference, we first examine elementary models for th scattering predicted from spin-density (i) and current-densit (ii) fluctuations for a normal Fermi liquid. We then conside fluctuations of the currents (iii) and of the lattice (iv) associate with the build up of short-range order. The results rule out th simplest treatments of these mechanisms for the observed scattering and point to possible ways forward.

In models (i), (ii) and (iii) the focus of attention is the coupling of the neutron magnetic moment with the local dynamical magnetic field $\mathbf{B}(\mathbf{r}, t)$ generated by fluctuations in the electron spin and current densities. The connection between the scattering cross-section and the power spectrum of the field fluctuations will be considered for an unpolarized beam and an isotropic body in a Schrödinger-Pauli model[1]. In this case the probability, per atom, incident flux, solid angle Ω and frequency ω, of neutrons being scattered from initial momentum $\hbar\mathbf{k}$ to final momentum $\hbar\mathbf{k}' = \hbar(\mathbf{k}+\mathbf{q})$ with energy increase $\hbar\omega$ can be expressed as

$$\frac{\mathrm{d}^2\sigma}{\mathrm{d}\Omega\,\mathrm{d}\omega} = \frac{\gamma^2 e^2 k'}{48\pi^3\hbar^2 c^2 nk}\langle|\mathbf{B}(\mathbf{q}, \omega)|^2\rangle \qquad (1)$$

where γ is the neutron gyroscopic factor (1.913), e is the electronic charge, c is the speed of light, n is the number of atoms per unit volume, $k = |\mathbf{k}|$, $k' = |\mathbf{k}'|$ and $\mathbf{B}(\mathbf{q}, \omega)$ is defined as $\nu^{-1/2}$ times the Fourier transform $\int \mathrm{d}^3r \int \mathrm{d}t\, \mathbf{B}(\mathbf{r}, t) \exp[-i(\mathbf{q}\cdot\mathbf{r} - \omega t)]$ of $\mathbf{B}(\mathbf{r}, t)$ defined over ν, the product of a large volume V and a long time interval. The quantity $\langle|\mathbf{B}(\mathbf{q}, \omega)|^2\rangle = \langle\mathbf{B}(-\mathbf{q}, -\omega)\cdot\mathbf{B}(\mathbf{q}, \omega)\rangle$ is the power spectrum equal to the Fourier transform of the unsymmetrized autocorrelation function $V^{-1}\int \mathrm{d}^3r'\langle\mathbf{B}(\mathbf{r}', 0).\mathbf{B}(\mathbf{r}'+\mathbf{r}, t)\rangle$.

The cross-section $\mathrm{d}\sigma/\mathrm{d}\Omega$ for total scattering in a given direction is obtained by integrating $\mathrm{d}^2\sigma/\mathrm{d}\Omega\,\mathrm{d}\omega$ over ω at fixed scattering angle with $\mathbf{q}$ (and ω) satisfying the conservation condition $\hbar\omega + \hbar^2k^2/2M = \hbar^2k'^2/2M$, $\hbar\mathbf{k}' - \hbar\mathbf{k} = \hbar\mathbf{q}$, where M is the neutron mass. The quantity plotted in Fig. 1 is the change of $\mathrm{d}\sigma/\mathrm{d}\Omega$ relative to a q-dependent background of ~10 barn steradian^{-1} atom^{-1}, averaged over the azimuthal angle of scattering. The use of low-energy neutrons and small scattering angles near the forward direction ensures that only slow fluctuations of long wavelength, which are those of special interest near T_c, contribute to the measured cross-section.

We may express the power spectrum of field fluctuations as the sum of two terms:

$$\langle|\mathbf{B}(\mathbf{q}, \omega)|^2\rangle = (4\pi)^2\frac{6\hbar\omega}{e^{\hbar\omega/k_BT}-1}\cdot\left[\frac{\mathrm{Im}\,\chi}{\omega} + K\frac{\mathrm{Re}\,\sigma}{c^2q^2}\right] \qquad (2)$$

The first is the familiar 'magnetic' or spin-scattering term which is most conveniently expressed in terms of the absorptive (imaginary) part of the magnetic spin susceptibility $\chi(\mathbf{q}, \omega)$. The second is the orbital or current fluctuation term which is proportional to the real (absorptive) part of the transverse conductivity. The factor $K = K(\mathbf{q}, \omega)$ is the dynamic screening factor, which is of decisive importance at very long wavelengths and low frequencies and is given by

$$K(\mathbf{q}, \omega) = \frac{\mu^2}{[1 - \mu\omega^2/c^2q^2 + 4\pi\omega\mu\,\mathrm{Im}\,\sigma/c^2q^2]^2 + [4\pi\omega\mu\,\mathrm{Re}\,\sigma/c^2q^2]^2} \qquad (3)$$

where $\mu = \mu(\mathbf{q})$ is the wavevector-dependent static magnetic permeability, and $\sigma = \sigma(\mathbf{q}, \omega)$ is the transverse conductivity with real and imaginary parts, $\mathrm{Re}\,\sigma(\mathbf{q}, \omega) + i\,\mathrm{Im}\,\sigma(\mathbf{q}, \omega)$ respectively. The two contributions (spin and current fluctuation) to the magnetic-field power spectrum can be readily estimated for the normal Fermi liquid state[3-8]. A full numerical analysis indicates that both mechanisms are at least two orders of magnitude too weak to account for the observed scattering near T_c.

As T_c is approached the Fermi liquid description is naturally expected to break down, and we now consider the extent to which the conventional description of this breakdown helps us to understand the enhanced scattering observed. The formation of virtual Cooper pair states, or more precisely the growth of short-range superconducting order, leads to a local enhancement of the conductivity at low frequencies and hence to a build up of slow fluctuations of the current density (case (iii)). The local conductivity is increased not only by the enhanced mobility of the virtual pairs, but also by an increased mobility of the unpaired carriers, whose motion near regions of emerging order is modified from that of the normal state. Current fluctuations arising from the enhanced mobility of virtual pairs in an isotropic state may be determined directly in terms of fluctuations of the complex order parameter $\psi(\mathbf{r}, t) = |\psi| \exp(i\theta)$, which yields the local pair concentration from $|\psi|^2$ and the local supercurrent density $\mathbf{J}_s(\mathbf{r}, t)$ for pairs of charge-to-mass ratio e/m through the relation $\mathbf{J}_s = \hbar e/2im(\psi^*\nabla\psi - \psi\nabla\psi^*)$. When the gradient of $|\psi|$ can be ignored, $\mathbf{J}_s$ reduces to $(\hbar e/m)|\psi|^2\nabla\theta$ and is therefore proportional to the local concentration of supercarriers and the local gradient of the phase $\theta(\mathbf{r}, t)$ of the order parameter. The current power spectrum $\langle|\mathbf{J}_s(\mathbf{q}, \omega)|^2\rangle$, and the corresponding conductivity and neutron scattering cross-section, can be directly evaluated from the statistical properties of $\psi(\mathbf{r}, t)$ normally described in terms of a time-dependent Ginzburg-Landau model. In the conventional analysis the fluctuations of $\psi(\mathbf{q}, t)$ are represented in terms of a gaussian stochastic process characterized by a single relaxation rate $\Gamma(\mathbf{q})$, expanded up to second order in $\mathbf{q}$, which softens at low $\mathbf{q}$ as T_c is approached[9,10].

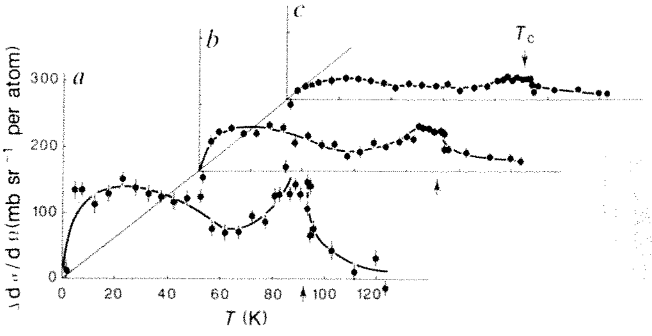


FIG. 1 Differential neutron scattering cross-section of high-homogeneity and high-purity polycrystalline samples of $YBa_2Cu_3O_{7-\delta}$ against temperature, in absolute units of millibarns per steradian per average atom. The measurements were made on the small-angle spectrometer D11 at the Institut Laue-Langevin, with incident wavelengths of 6.5 Å. In the three frames *a*, *b* and *c* the angles of scattering correspond to elastic scattering wave vectors of of 0.035 Å^{-1}, 0.045 Å^{-1} and 0.055 Å^{-1}, respectively. The cross-sections are averages over the azimuthal angle of scattering and are given relative to a q-dependent background level of ~10 barn steradian^{-1} atom^{-1}. Angular normalization and absolute calibration of the data has been carried out with the use of standard plexiglass and vanadium samples, respectively. The arrows indicate the value of T_c obtained from measurements of the bulk diamagnetic susceptibility.

Using this framework, a standard calculation yields a contribution to the conductivity that has qualitatively the correct temperature dependence (inverse power $(T - T_c)$). But if the parameters of the time-dependent Ginzburg-Landau model are taken to be those of the Bardeen-Cooper-Schrieffer theory for the electron gas, the cross-section is, like that of the normal state, far too weak to account for the observed scattering. This conclusion is not substantially changed when the enhanced mobility of the unpaired carriers is taken into account within existing frameworks[11-13].

So far we have considered only the coupling of the magnetic moment of the neutron to the fluctuating magnetic fields. The neutron also interacts directly with the nuclei (case (iv)). Because of the low incident energies and small scattering angles used, calculations based on the conventional analysis of lattice vibrations (single or multiple scattering involving zero, one or more phonons) cannot account for the observed intensity. Furthermore the observed fall in cross-section with increasing wavevector and temperature above T_c is not reproduced in such models.

The above considerations do not definitely rule out any of the three general scattering mechanisms (spin, current or nuclear). They merely indicate that the simplest forms of these mechanisms, within the limitations of the isotropic model considered, fail to give satisfactory quantitative agreement with the measured cross-sections. In the case of spin scattering, we may question the assumption of negligible susceptibility $\chi(\mathbf{q}, \omega)$: particularly for wavelengths shorter than the superconducting coherence length but longer than the spin correlation length, an enhancement of the susceptibility near T_c could affect both the spin and current scattering cross-sections and thus affect cases (i), (ii) and (iii). The second point of weakness arises in the usual analysis of supercurrent fluctuations, in which a phenomenological model is treated in a gaussian approximation with the parameters based on a mean-field theory and the electron-gas model, and in which the effects of interactions of the unpaired carriers with the fluctuations of the order parameter are incompletely developed. The indirect effects that short-range order near T_c has on the displacements of the atoms from their normal equilibrium position (case (iv)) also need further investigation; a complete analysis appropriate for the conditions of our experiments has not yet been carried out. Finally, there exist interpretations of the data, not considered here, that rely on detailed microscopic modelling, for example quasielastic scattering from fields of persistent currents due to vortex lines or superconducting clusters and, in particular, scattering due to fluctuations in the angular momentum of pairs in anisotropic states.

The anomalous scattering we have observed appears to be associated with the dynamics of the superconducting order parameter, but standard models still cannot give an elementary account of the magnitude of the scattering. At this stage it is unclear whether the effect is unique to high-T_c superconductivity, with its quasi-two-dimensional character and possibly exotic quasiparticles. To this end we have initiated a programme of study of more conventional superconductors. ☐

Received 18 January; accepted 15 March 1991.

1. Marshall, W. & Lovesey, S. W. *Theory of Thermal Neutron Scattering* (Oxford University Press, 1971).
2. Joynt, R. & Rice, T. M. *Phys. Rev.* B**38,** 2345–2353 (1988).
3. Bernheoft, N. R., Hayden, S. M., Lonzarich, G. G., Paul, D. McK. & Lindley, E. J. *Phys. Rev. Lett.* **62,** 657–660 (1989).
4. Bezinge, A., Jorda, J. L., Junod, A. & Muller, J. *Solid St. Commun.* **64,** 80–82 (1987).
5. Landolt-Börnstein *Magnetic Properties* vol. 2 (Springer, Berlin, 1962).
6. Martin, P. C. *Phys. Rev.* **161,** 143–155 (1967).
7. Sasaki, K. & Obata, Y. *Suppl. Prog. theor. Phys.* **69,** 406–419 (1980).
8. Lindhard, J. *Mat.-Fys. Meddr Kongelige danske Videnskabernes Selskabs* **28.8** (1954).
9. Aslamasov, L. G. & Larkin, A. I. *Phys. Lett.* A**26,** 238 (1968).
10. Schmidt, H. *Z. Phys.* **216,** 336–345 (1968).
11. Maki, K. *Prog. theor. Phys.* **39,** 897–906 (1968).
12. Thompson, R. S. *Phys. Rev.* B**1**, 327–333 (1970).
13. Maki, K. & Thompson, R. S. *Phys. Rev.* B**39,** 2767–2770 (1989).

ACKNOWLEDGEMENTS. We thank the staff at the Institut Laue-Langevin for their cooperation and a number of our colleagues, in particular Y. Chen, P. Coleman, U. Cox, M. Delap, M. Gunn, A. Huxley, H. Mook, G. Squires, W. Stirling and J. Waldram, for their interest and for helpful discussions. This study was supported by the SERC.

Low organic carbon accumulation rates in Black Sea sediments

S. E. Calvert*, R. E. Karlin†, L. J. Toolin‡,
D. J. Donahue‡, J. R. Southon§¶ & J. S. Vogel§¶

* Department of Oceanography, University of British Columbia,
Vancouver V6T 1W5, Canada
† Mackay School of Mines, University of Nevada, Reno,
Nevada 89557, USA
‡ NSF-Arizona AMS Facility, University of Arizona, Tucson,
Arizona 85721, USA
§ Department of Archaeology, Simon Fraser University, Burnaby,
British Columbia V5A 1S6, Canada

THE Black Sea, the world's largest anoxic marine basin, is frequently used as a modern analogue for the formation of organic-rich sediments and carbonaceous rocks[1–3], on the widely held assumption that anoxic conditions promote the preferential preservation of organic matter in sediments. Data for testing this hypothesis have so far been equivocal[4–7], but here we use radiocarbon ages obtained using accelerator mass spectrometry for the organic fraction of recent Black Sea sediments to estimate the organic carbon accumulation rates. These range from 0.69 to 2.09 g C m^{-2} yr^{-1} and are significantly lower than earlier estimates based on varve counting[6]. Depending on the value taken for the rate of primary production in the Black Sea[4,8], between 0.7 and 2.1% of the organic carbon is preserved in the bottom sediments. When compared with carbon accumulation rates in equivalent oxygenated environments[9], these results indicate that the modern Black Sea is not a site of anomalously high organic carbon accumulation. This suggests that anoxic conditions in the water column may not be a prerequisite for the preservation of organic matter in marine sediments, and that models of the origin of carbonaceous facies in the geological record may therefore need to be modified.

The Holocene section in the Black Sea[10] comprises a modern, finely laminated coccolith marl (unit 1) overlying a microlaminated organic-rich sapropel (unit 2) which, in turn, overlies moderately laminated calcareous clays (unit 3). The units are thought to reflect changes in sedimentation first from a Pleistocene freshwater lake (unit 3) to an early phase of the present anoxic, brackish basin (unit 2) formed when the Black Sea was reconnected with the Mediterranean by the Bosporus Strait after the post-glacial sea-level incursion, then to the present highly anoxic marine basin (unit 1).

The cores were collected during Leg 4 of the cruise of RV *Knorr* of the Woods Hole Oceanographic Institution in June–July 1988 (Fig. 1). We used a Kastengreifer[11] box-corer to collect undisturbed samples of the modern unit 1 sediments. Smaller sub-samples of the box cores were taken by inserting 10-cm-diameter butyrate core tubes into the boxes. Before removing the sub-cores from the boxes, we collected samples of the superficial 'fluff' layer[12] by carefully syringing off the upper few millimetres of the flocculent sediment from the undisturbed surfaces of some of the box cores. The sub-cores were processed by extruding and centrifuging successive 1- or 2-cm sections and then freeze-drying the solids. At each level, we also collected small sub-samples to determine water content.

In the shore laboratory, we carried out carbon analysis and radiocarbon dating on the sub-samples. The total and carbonate carbon values were determined by combustion-gas chromatography and by coulometry, with precisions of ±1.2 and 3.7% (2σ) respectively. Organic carbon was taken to be the difference between total and carbonate carbon. We calculated calcium carbonate contents from the carbonate carbon values assuming no other carbonate-bearing phase was present. Samples for radiocarbon dating were treated overnight with 10% HCl, washed three times with distilled water, dried and pulverized. The methods used for the radiocarbon assays of cores BS4-9BC and BS4-14BC at Arizona are given in refs 13 and 14, and those for core BS4-15BC at McMaster University in refs 15 and 16.

Figure 2 shows that the sediments recovered from unit 1 in the abyssal area of the Black Sea (cores BS4-9BC and BS4-14BC

¶ Present address: Lawrence Livermore National Laboratory, Livermore, California 94550, USA.

TABLE 1 Sedimentation rates, sediment properties and organic carbon accumulation rates

Core number	Water depth (m)	Sedimentation rate (cm per 1,000 yr)	Grain density (g cm^{-3})*	Porosity†	Total sediment accumulation rate (g m^{-2} yr^{-1})	Organic carbon (%)‡	Organic carbon accumulation rate (g C m^{-2} yr^{-1})
BS4-9BC	2,087	15.9	2.42	0.91	38.7	6.04	2.16
BS4-14BC	2,218	15.8	2.43	0.91	35.7	5.07	1.81
BS4-15BC	198	17.0	2.48	0.90	42.2	1.64	0.69

* Calculated assuming the following grain densities: $CaCO_3$ 2.7 g cm^{-3}; organic matter (organic carbon × 2) 1.0 g cm^{-2}; clay (1 − ($CaCO_3$ + organic matter)) 2.5 g cm^{-3}.

† Obtained from the water contents, corrected for the presence of salt in the dried samples. Mean values for the intervals below the marked decrease in organic C content (Fig. 1).

‡ Mean values for the intervals below the marked decrease in organic C content (Fig. 1).

have organic carbon contents that decrease from surface values of 9.6% to 4-7% at depths of 1 cm. Core BS4-15BC, recovered from a water depth of 198 m, has lower organic carbon contents. The radiocarbon ages of the organic fraction show that the surface 'fluff' samples are either essentially modern or contain bomb radiocarbon. Below the surface layers, the radiocarbon ages increase to ~3,000 years BP (before present) at the bases of the box cores. Evidence from gravity cores collected at the same sites indicates that the bottoms of the box cores were very close to the boundary between units 1 and 2. Regression plots of age against depth for the three cores (Fig. 2) have surface intercepts of 593-1,215 years BP, and slopes that yield sedimentation rates of 15.8-17.0 cm per 10^3 yr (Table 1).

The age of the unit 1/unit 2 boundary in the Black Sea has been estimated previously at 3,000 BP from conventional radiocarbon dating of bulk organic matter[10], and at 1,000 BP from varve counting[17]. The latter value was based on the assumptions that the fine-scale laminations in unit 1 are strictly annual deposits (varves)[18,19] and that the complete section is preserved. Degens *et al.*[17] argued that the radiocarbon ages are affected by old, presumably reworked, organic and/or carbonate carbon in the sediments, and that 2,000 years should be subtracted from ^{14}C ages to make them conform with the varve ages: in the absence of surface samples, this argument was accepted[5]. The new data for unit 1 (Fig. 2) show that the extrapolations of the age/depth curves to the surfaces of the cores yield ages that are substantially less than 2,000 years; a correction for the apparent radiocarbon age of the surface waters, probably lying between the value of 400 years adopted for the open ocean[20] and the value of 1,430 years estimated from the ^{14}C balance in the Black Sea[21], would lower these ages further. Hence, any contribution of old reworked carbon in these sediments is probably small; although some of the organic fraction of the unit 1 sediments may be derived from terrestrial sources[5], this material is not necessarily older than the sediments into which it is mixed. In addition, radiocarbon ages of coccolith carbonate from unit 1 sediments, derived by accelerator mass spectrometry[5,22] are similar to those of the organic carbon fraction. It seems unlikely that the admixture of old, reworked organic material and carbonate into the sediments would produce such similar radiocarbon ages for these fractions. We therefore interpret the agreement between carbonate and organic carbon ages as further evidence that there is very little old reworked organic or carbonate carbon in the unit 1 sediments, thus questioning the accuracy of the varve chronology.

Given the relative youth of the core surfaces as extrapolated from ^{14}C measurements, we assumed that the differences between the true and radiocarbon ages were sufficiently small and constant over the dated core intervals that the sedimentation rates derived from Fig. 2 were correct, and we used the radiocarbon dating to derive total sedimentation and organic carbon accumulation rates (Table 1). In these calculations, the organic carbon contents from below the organic-rich surface layer were adopted as the values most relevant to long-term burial. The total mass accumulation rates are 34.5-42.2 g m^{-2} yr^{-1} and the organic carbon accumulation rates range from 0.69 g C m^{-2} yr^{-1} at 198 m to 1.75-2.09 g C m^{-2} yr^{-1} at 2,000 m.

Measurement by ^{210}Pb determination[23] of the sedimentation

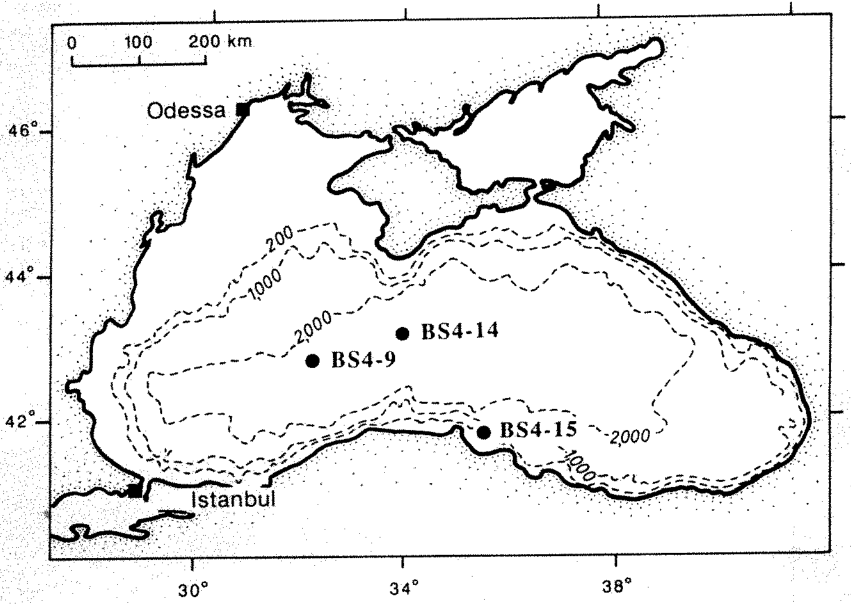


FIG. 1 Locations of the box cores used in this study. Isobaths are in metres.

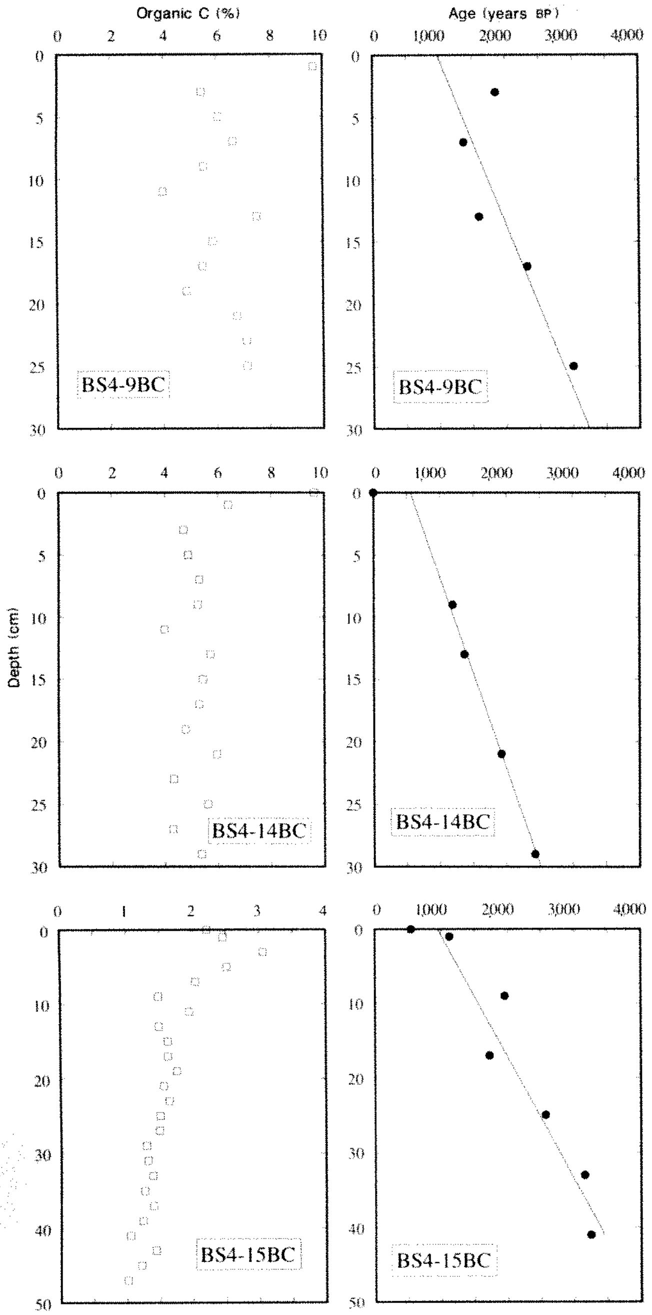


FIG. 2 Depth distributions of organic carbon and radiocarbon ages in three box cores from the Black Sea. Samples at a depth of 0 cm represent the 'fluff' layer. The surface 'fluff' sample in core BS4-14BC has been omitted from the regression for this core because it contains bomb ^{14}C.

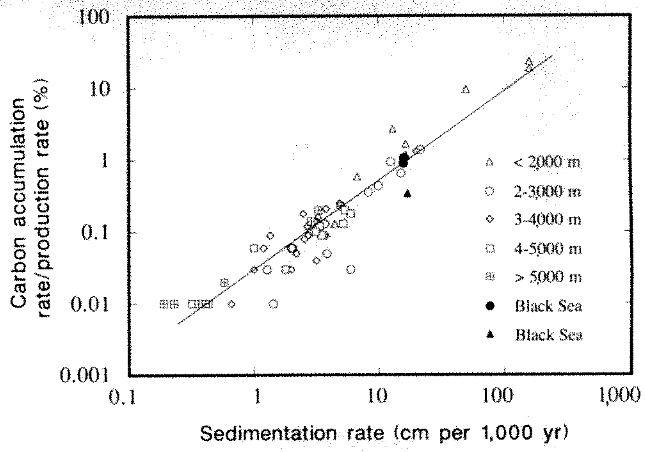


FIG. 3 Organic carbon accumulation rates normalized to primary productio rates (% carbon buried) as a function of sedimentation rates. Open symbol represent data from open marine, oxygenated environments and are take from ref. 29. The solid triangle represents data for core BS4-15, and th solid circles represent data for cores BS4-9 and BS4-14 (Table 1), a normalized to a primary production rate of 200 g C m^{-2} yr^{-1} (ref. 8). Adoptio of the primary production value assumed by Deuser[4] would change th position of the Black Sea data points by a factor of 2, but they would remai within the scatter around the regression line shown. The equation o regression line is $A = 0.028\,S^{1.2503}$, where A is the normalized accumulatio rate and S the sedimentation rate; the correlation coefficient $r^2 = 0.96$.

rate in the upper 3 cm of core BS4-9 yielded a total sediment accumulation rate of 55 g m^{-2} yr^{-1} which is compatible with the 34.6 g m^{-2} yr^{-1} determined by the radiocarbon method over a longer time interval. Our calculated organic carbon accumulation rates are similar to those of between 0.1 and 4.8 g C m^{-2} yr^{-1} reported[24] for water depths below 1,000 m. Furthermore, total mass accumulation rates of unit 1 sediments based on varve counting[6] range from 150 to 171 g m^{-2} yr^{-1}, and yield organic carbon accumulation rates of 6.0–7.0 g C m^{-2} yr^{-1}. These are higher than the settling fluxes of organic carbon (1.4–1.8 g C m^{-2} yr^{-1}) measured by moored sediment traps at two stations at 1,200-m depth in the Black Sea[19]. These data, together with the detailed comparison[23] between lamina counting and ^{210}Pb determinations, further indicate that the assumption of continuous deposition of strictly annual lamina couplets in thi basin may be incorrect.

The 'burial efficiency' of organic matter in marine sediment (the proportion of the organic carbon accumulating on th sediment surface that becomes permanently buried[25]) is a stron function of water depth and total sedimentation rate in oxygenated environments[9]. It is widely believed to be higher a equivalent sedimentation rates under anoxic conditions, but thi conclusion has been disputed[25-27]. Canfield[7] used data on th sulphate reduction rates in abyssal sediments from the Blac Sea[24] to derive the corresponding organic carbon oxidation rate which yielded very high burial efficiencies of 13–51% and thu supported the conclusion. An alternative way of estimating th degree of preservation of organic carbon in the sediments is t compare the accumulation rates with the primary productio rates in the overlying water column. A value for the primar production rate in the Black Sea of 100 g C m^{-2} yr^{-1} (ref. 4 would yield organic carbon burial rates for this basin of 0.7–2.1% (using the data in Table 1). But Sorokin[8] has argued that th primary production rate in the Black Sea is probably doubl this value because of the undersampling of the seasonal production maxima[18]; measurements of the primary production rat in the Black Sea in May 1988 (the period following the sprin bloom) of 0.575 g C m^{-2} d^{-1} (ref. 28) support this argumen Using this new information, the organic carbon burial rates i the deep water sediments of the Black Sea cannot be distinguished from those of oxic open-ocean sediments accumulatin at similar water depths and sedimentation rates (Fig. 3). W conclude that organic carbon accumulation rates in anoxic Blac Sea sediments have been overestimated in the past because c inaccurate chronologies; contrary to the commonly accepte view, the central and southern Black Sea is not a site c anomalously high organic carbon accumulation, and carbo burial rates are similar to those in the open ocean.

Received 14 November 1990; accepted 12 March 1991.

1. Woolnough, W. G. *Bull. Am. Ass. Petrol. Geol.* **21**, 1101–1157 (1937).
2. Thiede, J. & van Andel, T. H. *Earth planet. Sci.* **33**, 301–309 (1977).
3. Demaison, G. J. & Moore, G. T. *Org. Geochem.* **2**, 9–31 (1980).
4. Deuser, W. G. *Deep-Sea Res.* **18**, 995–1004 (1971).

5. Calvert, S. E., Vogel, J. S. & Southon, J. R. *Geology* **15,** 918–921 (1987).
6. Hay, B. J. *Paleoceanography* **3,** 491–508 (1988).
7. Canfield, D. E. *Deep-Sea Res.* **36,** 121–138 (1989).
8. Sorokin, Y. I. in *Ecosystems of the World, Vol. 26: Estuaries and Enclosed Seas* (ed. Ketchum, B. H.) (Elsevier, Amsterdam, 1983).
9. Müller, P. J. & Suess, E. *Deep-Sea Res.* **26,** 1347–1362 (1979).
10. Ross, D. A., Degens, E. T. & McIlvaine, J. *Science* **170,** 163–165 (1970).
11. Kögler, F-C. *Meyniana* **13,** 1–7 (1963).
12. Pilskaln, C. H. *Trans. Am. Geophys. Un.* **71,** 151 (1990).
13. Slota, P. J., Jull, A. J. T., Linick, T. W. & Toolin, L. J. *Radiocarbon* **29,** 303–306 (1987).
14. Linick, T. W., Jull, A. J. T., Toolin, L. J. & Donahue, D. J. *Radiocarbon* **28,** 522–533 (1986).
15. Vogel, J. S., Southon, J. R., Nelson, D. E. & Brown, T. A. *Nucl. Instrum. Meth.* **233,** 289–293 (1984).
16. Nelson, D. E., Vogel, J. S., Southon, J. R. & Brown, T. A. *Radiocarbon* **28,** 215–222 (1986).
17. Degens, E. T. *et al. Neues Jb. Geol. Paläontol. Mh.* 1980, 65–86 (1980).
18. Honjo, S. *et al. Mitt. Geol. Paläontol. Institut Univ. Hamburg* **62,** 19–39 (1987).
19. Hay, B. J. *et al. Deep-Sea Res.* **37,** 911–928 (1990).
20. Broecker, W. S. & Peng, T-H. *Tracers in the Sea,* 690 (Eldigeo, Palisades, New York, 1982).
21. Murray, J. W., Top, Z. & Ozsoy, E. *Deep-Sea Res.* (in the press).
22. Jones, G. A. *Trans. Am. Geophys. Un.* **71,** 152 (1990).
23. Crusius, J. & Anderson, R. *Paleoceanography* (submitted).
24. Vaynshteyn, M. B., Tokarev, V. G., Shakola, V. A., Lein, A. Y. & Ivanov, M. V. *Geokhimiya* **7,** 1032–1044 (1985); (Engl. transl.) 110–122.
25. Henrichs, S. M. & Reeburgh, W. S. *Geomicrobiol. J.* **5,** 191–237 (1987).
26. Pedersen, T. F. & Calvert, S. E. *Bull. Am. Ass. Petrol. Geol.* **74,** 454–466 (1990).
27. Calvert, S. E. & Pedersen, T. F. in *Productivity, Accumulation and Preservation of Organic Matter in Recent and Ancient Sediments* (eds Whelan, J. K. & Farrington, J. W.) (Columbia University Press, in the press).
28. Karl, D. M. & Knauer, G. A. *Deep-Sea Res.* (in the press).
29. Sarnthein, M., Winn, K., Duplessy, J-C. & Fontugne, M. R. *Paleoceanography* **3,** 361–399 (1988).

ACKNOWLEDGEMENTS. Participation in the cruise of RV Knorr was supported by the Natural Sciences and Engineering Research Council of Canada (S.E.C.) and the NSF (R.E.K.). The help of cruise participants in retrieving the cores is gratefully acknowledged. Support for the McMaster University and University of Arizona AMS facilities was provided by the NSERC and NSF, respectively.

Prospects for eruption prediction in near real-time

Barry Voight* & Reinold R. Cornelius

College of Earth and Mineral Sciences, Pennsylvania State University,
University Park, Pennsylvania 16802, USA
* Also at US Geological Survey, Igneous & Geothermal Processes,
345 Middlefield Road, Menlo Park, California 94025, USA

THE 'materials science' method for eruption prediction[1–3] arises from the application of a general law governing the failure of materials: $\dot{\Omega}^{-\alpha}\ddot{\Omega} - A = 0$, where A and α are empirical constants, and Ω is an observable quantity such as ground deformation, seismicity or gas emission. This law leads to the idea of the 'inverse-rate' plot, in which the time of failure can be estimated by extrapolation of the curve of $\dot{\Omega}^{-1}$ versus time to a pre-determined intercept. Here we suggest that this method can be combined with real-time seismic amplitude monitoring to provide a tool for near-real-time eruption prediction, and we demonstrate how it might have been used to predict two dome-growth episodes at Mount St Helens volcano in 1985 and 1986, and two explosive eruptions at Redoubt volcano in 1989–90.

The materials science method may be applied to data sets from appropriately designed and located geodetic monitoring systems, for example from repeated electronic distance or tilt measurements. The method should be most useful, however, for seismic data, because seismicity is the phenomenon most commonly monitored to predict volcanic eruptions[1,4–10]. Characteristics of the seismic activity of volcanoes include the number of earthquakes, their energy and moment, and the seismic strain release. The number of earthquakes is less fundamental than their energy content.

Seismic energy calculations conventionally require study of records, recognition and classification of discrete earthquakes, and application of correlations involving energy-magnitude or energy-coda relations. Some of these operations may be computerized, but the conventional approach is manual and time-consuming, and energy calculations may lag the actual events by hours or days. Although useful, prediction on the basis of seismic energy is frequently not real-time.

During periods of intense seismicity, earthquake traces may overlap or be superposed on tremor of significant amplitude; individual earthquakes cannot be accurately counted, and coda lengths cannot be measured. Use of more distant seismic stations minimizes the noise due to tremor and small earthquakes, but introduces bias towards large-magnitude events and systematic error into estimates of coda magnitude[11].

These problems can be minimized by continuous measurement of the mean amplitude of seismicity[11–15]. The real-time seismic amplitude measurement (RSAM) system used by the US Geological Survey provides information from up to eight telemetered seismic stations[16]. The absolute voltage (amplitude) level for each station is digitized, averaged and appended in near real-time (averaging is over 1- or 10-min intervals) to a

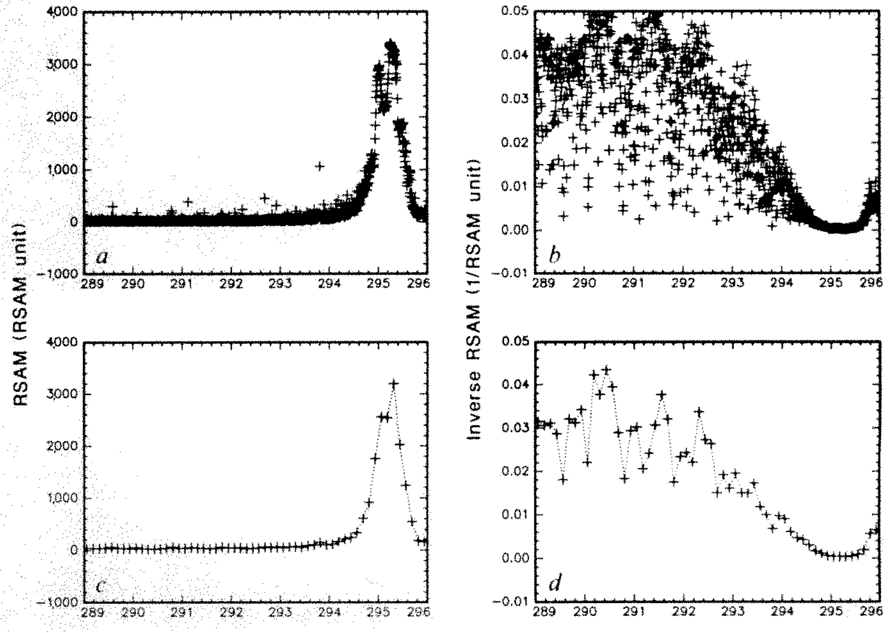


FIG. 1 RSAM from seismometer 'Garden', Mount St Helens, between 16 October (day 289) and 22 October 1986 (day 296). Time used is GMT. *a*, RSAM averaged over periods of 10 min. *b*, Inverse RSAM calculated for *a*. *c*, RSAM averaged over periods of 3 h. *d*, Inverse-RSAM calculated for *c*.

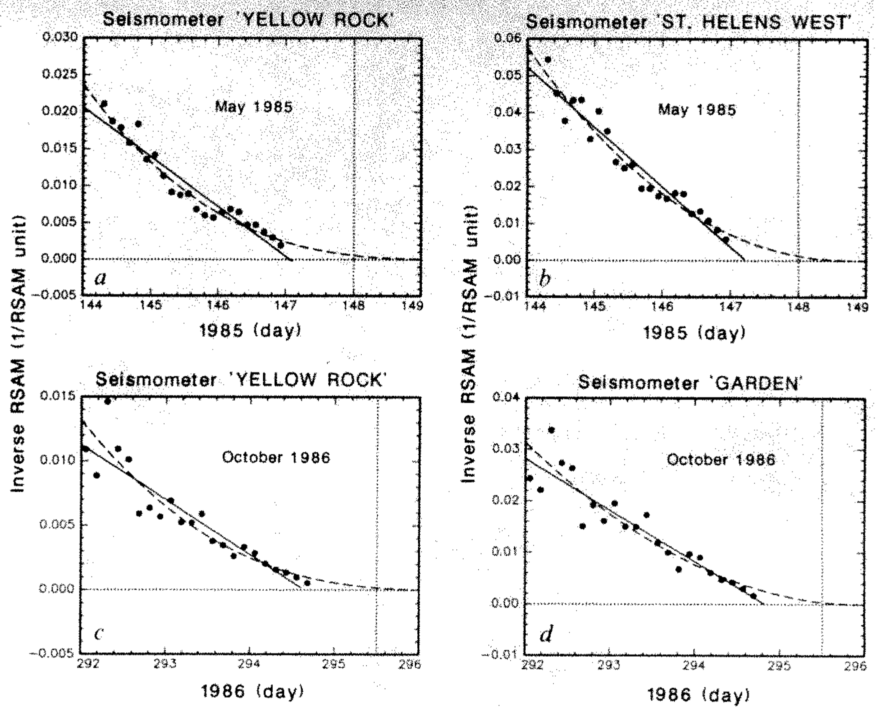


FIG. 2 Inverse-RSAM from three stations for the May 1985 and October 1986 eruptions at Mount St Helens. Data are averaged over 3 h. Station Yellow Rock is in the crater, 1 km from the dome; Garden, 500 m north of base of dome; St Helens West, 4 km outside the crater. Time used is GMT. *a, b*, Data from 1985 are for Yellow Rock and St Helens West between 24 May (day 144.3) and 27 May (day 147.0). *c, d*, Data from 1986 for Yellow Rock and Garden between 19 October (day 292.0) and 21 October (day 294.7). Data sets for the same eruption show similar features. Solid lines are fits to the data with $\alpha = 2$; dashed lines are fits by numerical methods with $1 < \alpha < 2$. Data sets are terminated roughly at the time of the point of inflection of the RSAM curve. Vertical lines represent approximate time of observed onset of surface events (pervasive dome disruption) and/or eruption.

data file on a multi-user computer system. A recent modification of the seismic-aquisition system used to monitor Redoubt Volcano, Alaska, allows average amplitudes in each of 16 spectral bands to be computed from digitized analog signals with a fast fourier transform (FFT)[16]. This has been termed SSAM (seismic spectral amplitude measurement)[17]. Such measurements, conventional or spectrally bracketed, provide a refined means of defining the relative intensities of eruptive episodes regardless of the type of activity (discrete earthquake, closely-spaced or overlapping earthquakes, or tremor).

If transducer output is known accurately, then each 1-min average can be scaled to give average ground displacement. This measurement can be adjusted for geometric spreading and instrument amplification to obtain the 'reduced displacement'[18]. Thus RSAM is directly proportional to commonly measured earthquake parameters such as moment rate or energy rate, as these also are proportional to reduced displacement.

Even if the proportionality constants are not precisely known, in terms of the materials science prediction method the RSAM output is directly analogous to the rate of energy or moment. The procedure of plotting inverse rate ($\dot{\Omega}^{-1}$) against time (t) may therefore be applied simply by inverting RSAM without further data manipulation.

Here we present examples for two distinctive styles of eruption involving Mount St Helens, Washington, in 1985 and 1986, and Redoubt volcano, Alaska, in 1989–90. Seismic activity associated with three growth episodes of the composite dacite dome at Mount St Helens produced very similar precursory RSAM

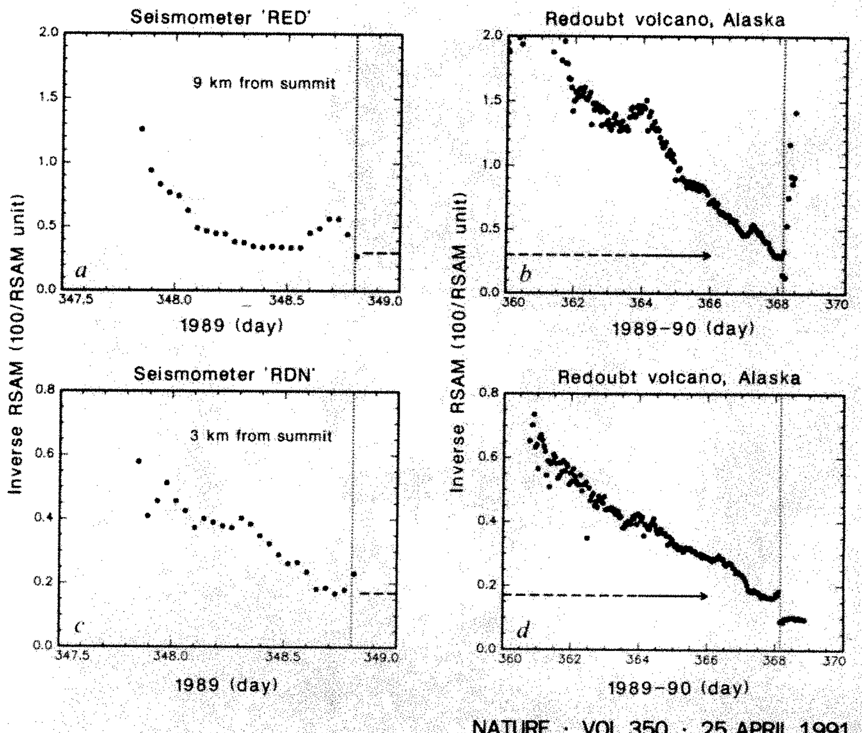


FIG. 3 Inverse-RSAM for stations RED (upper) and RDN (lower) at Redoubt volcano, before two major eruptions in 1989–90. Time used is GMT. Station RDN used automatic gain control, which may have influenced the measurements. *a, c*, The 1989 explosion of 14 December at 10:13 Alaskan Standard Time, AST (day 348.80), which resulted in an eruption plume 33,000 feet high. *b, d*, The 2 January 1990 vent-clearing eruption, which consisted of two explosive events at 17:48 and 19:27 AST (day 368.12 and 368.19); each resulted in a plume 40,000 feet high. Times of eruptions are marked as vertical dotted lines. A finite 'failure rate' (horizontal arrows) could have been estimated from plots for the 14 December eruption and the intercept with the inverse-RSAM curve in January used to predict time of eruption.

curves[11,19], with curvature typically $1 < \alpha < 2$, consistent with the materials science theory. The RSAM change is not exponential as previously assumed[11] (exponential growth implies $\alpha = 1$, but the best fits to the RSAM curves have $\alpha \neq 1$).

The last of these dome-building events involved endogenous growth and lava extrusion on 22 October 1986 (day 295)[19]. Figure 1 shows RSAM growth curves for both 10-min and 3-h average amplitudes monitored at one station near the dome. Also shown are the inverse-RSAM curves, a cornerstone of the materials science approach. Failure is indicated when a critical inverse value $\dot{\Omega}_f^{-1}$ is obtained; if $\dot{\Omega}_f^{-1}$ is uncertain, an approximate upper-bound failure date can be obtained from the point of intersection of the extrapolated inverse-rate curve with the time axis (this singularity occurs for $\alpha > 1$).

Figure 1 illustrates the reduction in scatter when amplitudes are averaged for periods exceeding an hour. Graphical extrapolations of inverse-RSAM before day 292 indicated that an event should be expected after day 296, but the processes associated with RSAM accelerated after day 292 leading to an earlier prediction date. Subsets of inverse-RSAM data after day 292 and similar data for the May 1985 eruption (Fig. 2) demonstrate the consistency of the method as observed over a broad network of seismic stations. Linear (assumed $\alpha = 2$) and curved ($1.2 < \alpha < 1.6$) extrapolations are shown; although these curves are perturbed by the small RSAM shift one day before eruption, the predictive capability of the inverse-RSAM procedure seems obvious. The onset of precursory RSAM acceleration varied at Mount St Helens from ~5 days before the October 1986 event (Fig. 1) to ~12 and 7 days, respectively, before the May 1985 and May 1986 events.

In practice, graphical inspection of the inverse-RSAM pattern as it approaches the abscissa is more useful than trying to predict events from the empirical constants. For $\alpha < 2$, the inverse-rate curves have a concave upward bend; linear extrapolation thus yields a lower-bound prediction.

On 14 December 1989, Redoubt volcano (Alaska) erupted after 23 years of quiescence[20]. Seismic activity was tracked by the RSAM system to provide consecutive 10-min averages of the absolute amplitude. Spectral analysis[17] showed that the main seismic excitation was in the range 1-2.5 Hz. For the largest eruptions the characteristic signals were observed 20 km from the summit; for the smaller events, near-source seismographs were required. Clear RSAM signals were seen before six of the eight significant eruptions that occurred between 5 March and 21 April, when at least one seismograph within 3 km of the summit was operating continuously.

Accelerations of RSAM amplitude also preceded the powerful vent-clearing phreatomagmatic explosions of 14-22 December and the dome-destroying explosions of 2 January AST (day 368). The 2 January explosions triggered a series of pyroclastic flows which in turn generated hot lahars and hyperconcentrated floods[20]. Precursory changes in RSAM for the 14 December event developed over little more than one day (Fig. 3*a* and *c*), whereas the pattern before 30 December (day 364) warns of eruption several days in advance. The inverse-RSAM 'failure rate' ($\dot{\Omega}_f^{-1}$) experienced in December (Fig. 3*a* and *c*) provides a reasonable guide to predict eruption on 2 January (note intersection of horizontal arrow with inverse-RSAM curves, Fig. 3*b* and *d*). This procedure is an alternative to the extrapolation of inverse-RSAM to the time abscissa (which would imply infinite rate), although it must be recognized that precursory processes (and hence critical RSAM values) may vary from event to event. Further study of this important issue is required.

Obviously, it is easier to recognize trends in hindsight with data sets complete to the date of eruption (Fig. 3), than it is to interpret real-time data as it arrives. As an example, we reconstruct in Fig. 4 sequential 'snapshots' of inverse-RSAM data, averaged hourly, before the 2 January eruption. We show analyses for five successive times between 25 December and 1 January, using the assumption $\alpha = 2$ (linear extrapolation) with the latest data (circles). These analyses have been produced in hindsight, as the method was not used at Redoubt in 1989. In Fig. 4, inverse-RSAM extrapolations after 29 December are all consistent with eruption on 2 January. Analysis for 27 December warned of the possibility of an early eruption, but from a trend shift that occurred shortly afterwards, we could have revised the interpretation.

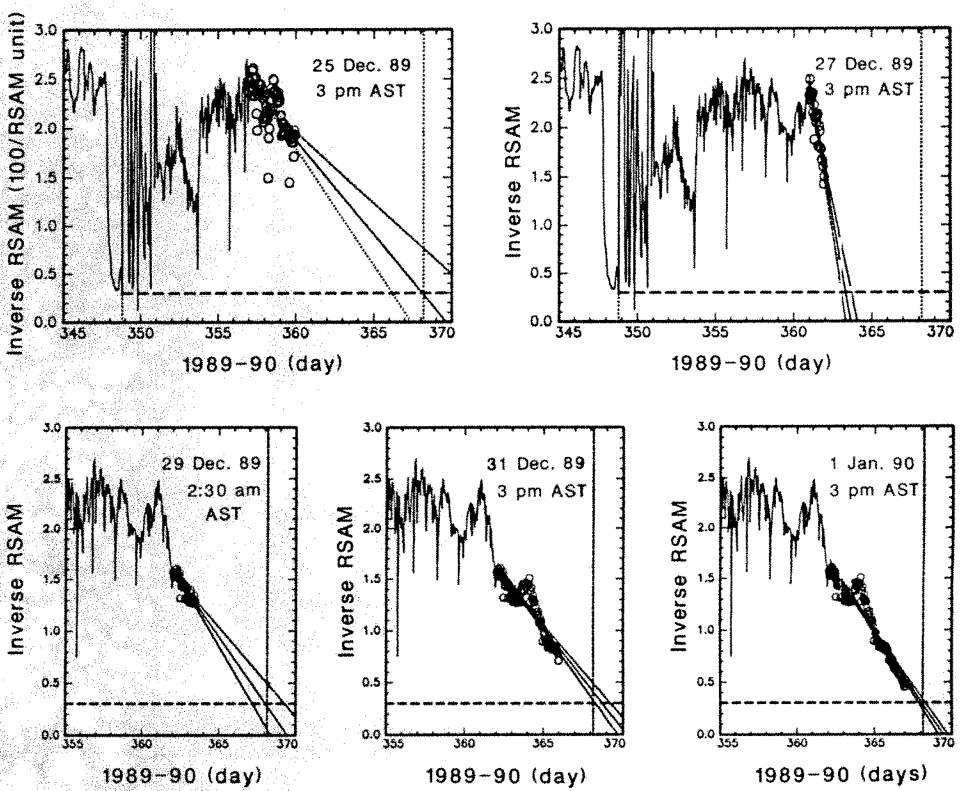


FIG. 4 Sequential analyses from station RED before eruptions on 2 January 1990. This sequence simulates analyses which might have been made as the time of eruption approached, although no inverse-rate analyses were actually performed in 1989. The eruptions on 14 December and 2 January are shown as vertical dotted lines; finite 'failure rate', as estimated from the December eruption, is shown as horizontal dashed line. Data are averaged over 1 h. Data used for analyses are shown as circles, other data as continuous lines. Analyses consist of linear least squares fits, assuming $\alpha = 2$, with an error envelope at the 97.5% confidence level. Bounds are set on the time of eruption by the intersection of linear projections and their envelopes with the estimated finite 'failure rate' (lower bound) and/or a possible infinite rate (upper bound). Analysis on 27 December (six days before eruption) might have led to a false early warning, but the trend changed shortly afterwards, and subsequent analyses suggested fairly accurate eruption times.

The examples presented here indicate that the method could provide a useful guide to prediction of eruptions, although the reliability of the method may be influenced by the regularity of structural mechanisms and loading conditions. Sharp breaks in observed rate with time, such as for Redoubt volcano (Fig. 4), may warn of changes in loading or structure. If the data imply RSAM change that is not (at least approximately) smooth and continuous, segmented analysis may be necessary. In such an approach, the initial value should be reassigned to exclude portions of the data set no longer considered applicable (Figs. 2 and 4). To apply the theory of this paper blindly, irrespective of such indications of change, is to misapply it.

A limitation of the method is the incorporation of seismic noise into the amplitude calculations, but misinterpretation can be minimized by comparing RSAM with (for example) gas, deformation or meteorological information, plotted on the same time base, and by studying analog seismic records or spectral data to identify tectonic earthquakes, fumarolic noise, storm noise and kindred phenomena[11]. We recognize that this method, in common with all known eruption prediction methods, will not necessarily work for all volcanoes, under all circumstances[3,10]; of 23 small explosive events at Tokachi-dake, Japan in 1988–89, only a third had common immediate precursors[15]. Predictive capability is best achieved by using a combination of data sets and methods, rather than by reliance on any single procedure. □

Received 9 October 1990; accepted 8 March 1991.

1. Voight, B. *Nature* **332,** 125–130 (1988).
2. Voight, B. *Science* **243,** 200–203 (1989).
3. Tilling, R. I. *Nature* **332,** 108–109 (1988).
4. Tokarev, P. I. *Bull. Volcan.* **35,** 243–250 (1971).
5. Tokarev, P. I. in *Forecasting Volcanic Events* (eds Tazieff, H. & Sabroux, J.-C.) Ch. 9 (Elsevier, Amsterdam, 1983).
6. Shimozuru, D. in *The Surveillance and Prediction of Volcanic Activity,* 19–45 (UNESCO, Paris, 1972).
7. Minakami, T. in *Physical Volcanology* (eds Civetta, L., Gasparini, P., Luongo, G. & Rapollo, A.) 313–333 (Elsevier, Amsterdam, 1974).
8. Malone, S. *et al. Science* **221,** 1376–1378 (1983).
9. Swanson, D. A. *et al. J. Geodynam.* **3,** 397–423 (1985).
10. Decker, R. W. *Ann. Rev. Earth planet. Sci.* **14,** 267–291 (1986).
11. Endo, E. T. & Murray, T. *Bull. Volcanology* (in the press).
12. Sassa, K. *Mem. Coll. Sci. Kyoto Univ.* **19,** 11–56 (1936).
13. Schick, R. *et al. J. Volcan. geotherm. Res.* **14,** 261–279 (1982).
14. Nishi, K. *Annls Disaster Prev. Res. Inst. Japan* B **30,** 1–18 (1990).
15. Okada, H. *Bull. Volcan. Soc. Japan* **35,** 175–203 (1990).
16. Murray, T. & Endo, E. T. *US geol. Surv. Open File Rep.* 89–684 (1989).
17. Stephens, C. D. *et al. EOS* (in the press).
18. Fehler, M. *J. geophys. Res.* **88,** 3476–3484 (1983).
19. Endo, E. T., Dzurisin, D. & Swanson, D. A. in *Magma Transport and Storage* (ed. Ryan, M. P.) Ch. 15 (Wiley, Chichester, 1990).
20. Alaska Volcano Observatory *EOS* **71,** 265–275 (1990).

ACKNOWLEDGEMENTS. We thank colleagues and US Geological Survey staff for assistance, and S. Malone, T. Murray, J. Power and R. I. Tilling for constructive criticism of the manuscript. This work was conducted under a cooperative project involving Penn State University, the Cascade Volcano Observatory (US Geological Survey) and the Alaska Volcano Observatory (US Geological Survey, University of Alaska Geophysical Institute and the Alaskan Division of Geological and Geophysical Surveys). Support was provided by NSF.

A fossil smut fungus from the anthers of an Eocene angiosperm

R. S. Currah & R. A. Stockey

Department of Botany, University of Alberta, Edmonton,
Alberta, Canada T6G 2E9

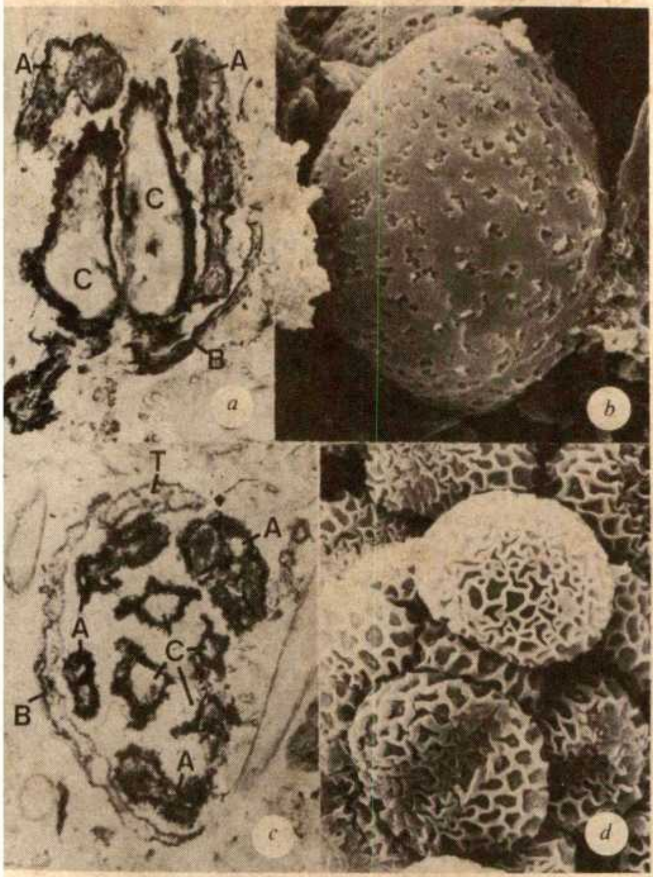
WHILE studying the floral morphology of a permineralized angiosperm from a Middle Eocene chert near Princeton, British Columbia, we encountered well-preserved masses of fungal spores in otherwise apparently normal anthers. The spore masses, which replaced the pollen, consist of single-celled, irregularly shaped to ovoid, minutely pitted spores. On the basis of intact nature of infected anthers and size, ornamentation and disposition of spores within anther locules, we have identified this material as an anthericolous smut fungus. This unequivocal fossil of the Ustilaginales shows that at least 48 million years ago the order had representatives with anthericolous sori, a characteristic of some extant smut taxa that permits effective insect dispersal of the fungus among populations of specific host plants.

The Ustilaginales includes about 1,000 species of obligate parasites that attack over 75 families of flowering plants, particularly the graminoid families, Cyperaceae and Poaceae[1]. As biotrophic parasites, they draw nutrients from plants without seriously affecting the reproductive potential of host species. Smut fungi reproduce in their angiosperm hosts by forming masses of thick-walled teliospores in sori which develop within the tissues and beneath the epidermis of leaves, stems, roots, ovaries, anthers and so on. Rupture of overlying epidermal tissue with exposure of a dusty excrescence of darkly pigmented spores is often the only indication that a plant is infected.

About 80 small flowers 0.9-mm long and 0.8 mm in diameter have been found in the Princeton chert (Fig. 1*a* and *c*). Twelve were examined for pollen by scanning electron microscopy. All specimens show masses of fungal spores, about 8 × 6 μm (Fig. 1*b*) and lack pollen in the anthers. The bilateral symmetry and the broken bases of the flowers suggest that they are borne on spikes. They possess a small receptacle, no disc, one to two perianth segments, four basifixed anthers opening by longitudinal slits, stamens free from corolla, non-connate filaments, four free carpels, a unilocular ovary, one ovule per locule and a single-seeded fruit with an aerenchymatous endocarp[2,3]. These characters suggest affinities with one of several aquatic monocotyledonous families in the Alismatidae, particularly the Aponogetonaceae, a small family native to the Old World Tropics, Australia and South Africa[4,5]. The fossil flowers, however, have four stamens rather than the six or more of the Aponogetonaceae. Pollen morphology, one of the most important characters used in identification of fossil flowers, is lacking because of the presence of teliospores. Formal taxonomic description of these flowers will be published elsewhere.

Extant smut taxa of Alismatidae are included in the genera *Doassansia*, *Burrillia* and *Tracya*[6]. These differ from the fossil material in producing teliospores in spore balls that develop in sori within leaves, petioles and stems. There are a few records of anthericolous smuts in extant Liliaceae[7], but none apparently for extant alismatidaceous taxa.

The presence of systemic biotrophic fungi in some angiosperms can cause abnormal developmental processes in flowers[8]. The effects of the smut fungus on floral development may explain the incongruous number of stamens in our fossil material. For example, dioecious plants of *Silene* infected with *Microbotryum violacea* often seem to have perfect flowers because the smut causes the staminodes of female flowers to develop into stamens, the anthers of which contain teliospores rather than pollen. Smutty anthers are reported to be as attractive to pollinators as normal anthers, so anthericolous smuts are assured of dispersal among their particular host species[9]. Identification of extant species and genera within the Ustilaginales is based on colour, size and shape of spores and spore balls, spore wall ornamentation, which varies from smooth to pitted or reticulate (Fig. 1*d*) to verrucose, the identity of the host plant and the mode of formation of basidiospores, which develop directly on a germ tube arising from a germinating teliospore. Without observations on the mode of basidiospore formation, it may be impossible to place the fossil smut in a modern genus. But until recently,



IG. 1 *a*, A longitudinal section showing carpels and stamens of the pernineralized Eocene flower. A, anther in which the smut sorus has replaced he pollen. B, bract. C, carpel. Magnification, ×42. *b*, Scanning electron nicroscopy of an individual, single-celled, minutely pitted, fossil teliospore vithin the sorus. Magnification. ×5,780. *c*, Transverse section of the flower. \, anther. B, bract. C, carpel. T, tepal. Magnification, ×60. *d*, Scanning electron nicroscopy of pitted teliospores within a sorus on an ovary of *Scorzonera umilis* L. infected with the extant smut *Ustilago scorzonerae* (Albertini and chweinitz) Schroeter. Magnification. ×2,720.

nost smuts forming simple, small (4–18-μm diameter) single-pored teliospores in the reproductive organs of angiosperms vere placed in the genus *Ustilago*[1,10]. The genus has now been livided into a number of smaller genera[6]. Of these, the genus *Microbotryum* is the closest to the fossil representative, with espect to teliospore morphology and sorus location. *Microotryum violaceum* (Persoon: Persoon) Deml and Oberwinkler = *Ustilago violacea* (Pers.: Pers.) Roussel) forms sori of single, lobose to somewhat irregular, pale lilac, finely and irregularly eticulate spores within the anthers of Caryophyllaceae.

The origin and evolution of the Ustilaginales are problematic ecause of the absence of any confirmed fossil material[11]. The ew references to their fossil record are based on questionable nterpretations of what might be a mixed collection of isolated eliospores from Ustilaginales and Uredinales in Tertiary ligites[11,12]. On the basis of the intact antheridial sori and the norphology of the teliospores, our material represents fossils f the Ustilaginales and indicates that the highly specialized lispersal mechanism of some modern representatives of the rder had evolved by at least 48 million years ago. □

---

eceived 11 December 1990; accepted 12 February 1991.

1. Fischer, G. W. *Manual of North American Smut Fungi* (Ronald, New York, 1953).
2. Cevallos-Ferriz, S. R. S. thesis, University of Alberta (1987).
3. Cevallos-Ferriz, S. R. S. & Stockey, R. A. *Rev. Palaeobot. Palynol.* (in the press).
4. Cronquist, A. *An Integrated System of Classification of Flowering Plants* (Columbia University Press, 1981).
5. Lawrence, G. H. M. *Taxonomy of Vascular Plants*. (Macmillan, New York, 1952).
6. Vanky, K. *Cryptogamic Studies* **1**, 1–159 (1987).
7. Lindeberg, B. *Symb. Bot. Upsal.* **16**, 1–175 (1959).
8. Clay, K. in *Coevolution of Fungi with Plants and Animals* (eds Pirozynski, K. A. & Hawksworth, D. L.) 79–105 (Academic, 1988).
9. Jennerston, O. *Oikos* **51**, 163–170 (1988).
10. Duran, R. in *The Fungi* (eds Ainsworth, G. C., *et al*) 281–300 (Academic, 1973).
11. Pirozynski, K. A. *A. Rev. Phytopath.* **14**, 237–246 (1976).
12. Tiffney, B. H. *Occasional Pap. Farlow Herbarium Cryptogam. Bot.* **7**, 1–42 (1974).

Perceptual filling in of artificially induced scotomas in human vision

V. S. Ramachandran & R. L. Gregory*

Psychology Department 0109, University of California, San Diego, La Jolla, California 92092-0109, USA
* Department of Psychology, University of Bristol, Bristol, UK

PATIENTS with scotomas or blind-spots in their visual field[1–5] resulting from damage to the visual pathways often report that the pattern from the rest of the visual field 'fills in' to occupy the scotoma. Here we describe a novel technique for generating an artificial perceptual scotoma which enabled us to study the spatial and temporal characteristics of this filling-in process. A homogeneous grey square subtending 1.5° was displayed against a background of twinkling two-dimensional noise of equal mean luminance (Fig. 1). On steady eccentric fixation for 10 s the square vanished and was filled in by the twinkling noise from the surround. Using this display we found that 'filling in' is an active visual process that probably involves creating an actual neural representation of the surround rather than merely ignoring the absence of information from the scotoma; filling in can occur separately for colour and texture, suggesting separate mechanisms; the filling-in process does not completely suppress information from the scotoma, even after an image has faded completely from consciousness it can nevertheless contribute to motion perception; and the process can be strongly influenced by illusory contours.

Patients with discrete lesions in their retinas or in primary visual cortex usually have a 'blind spot' or scotoma in their visual field as measured by conventional perimetry[1–5]. Oddly enough, if the patient gazes at a companion seen against a background of wallpaper, the companion's head may disappear but the gap gets 'filled in' by the surrounding wallpaper. It is said that King Charles II used to decapitate his courtiers using this benign procedure, although he used his natural blind spot rather than a scotoma.

Of the natural blind spot (corresponding to the optic nerve head), Sir David Brewster[6] has written: "We should expect, whether we use one or both eyes, to see a black or dark spot upon every landscape within 15° of the point which most particularly attracts our notice. The Divine Artificer, however, has not left his work thus imperfect... the spot, in place of being black, has always the same colour as the ground."

We know surprisingly little about the nature of the neural representation that corresponds to the filling in of scotomas and blind spots. To study the spatial and temporal characteristics of this process we created a novel stimulus that results in a temporary reversible scotoma in normal individuals[7,8]. A homogeneous grey square subtending 1.5° × 1.5° was displayed against a background of dynamic twinkling noise and the subjects were asked to fixate steadily on a spot 6° to the right (or left) of the square (Fig. 1). The twinkle had the same mean luminance (25 cd m^{-2}) as the square. Four naive subjects were used in all the experiments described here. After a brief period of steady fixation they reported that the square vanished completely and was filled in by the surrounding twinkle. The average time for fading and filling in was 5.04 s ($n = 40$; four subjects, 10 trials each.)

Does this filling-in process involve creating an actual neural representation of the 'twinkle' in the brain areas to which the grey square projects, or does it merely involve ignoring the absence of information? (After all, one is unaware of the huge gaping hole behind one's head, but one would not want to conclude, for example, that the wallpaper had been filled in behind the head.) One hint comes from the fact that the filling-in process often appeared gradual; after the square's borders faded, the twinkle filled in from outside to inside slowly, taking 2 or 3 s. This suggests an active process.

Is filling-in a unitary process or can it occur separately for colour and texture? To find out, we modified Fig. 1 so that the surround consisted of sparse black dots twinkling on a homogeneous pink background, and the square consisted of black spots moving horizontally against a grey background (Fig. 2) that was equiluminous with the pink. This square, defined by a difference in motion as well as colour, also faded completely in a few seconds, but we found that the filling-in occurred in two distinct stages. First, the homogeneous grey region in the square vanished and was filled in by the pink from the surround, so that one now had the experience of seeing the black dots moving against the filled-in pink that did not actually exist on the retina. Once this had occurred, the moving spots also faded and were replaced a few seconds later by the twinkling spots in the surround. Thus, there may be separate fill mechanisms for colour and texture corresponding, perhaps, to the different extrastriate visual areas[9-12] that are thought to be specialized for colour or motion (twinkle). Perhaps the colour border fades first and the surround colour is then assigned to this region (in the 'colour areas' such as V4). Because the moving spots have not yet faded, the visual system 'assumes' that the spots must be moving against the filled-in pink.

Does the fading of the square (and subsequent filling in) depend mainly on the adaptation of neural detectors that extract borders of the square or of the homogeneous region inside it? To answer this question we allowed the filling in to occur and then suddenly switched off and replaced the square with a slightly smaller concentric square (1.3°×1.3°). Because the region corresponding to this small square has already been filled in with twinkle, does one have to start the filling in again? The answer is yes. The small square did become clearly visible and took several seconds to fade and become 'filled in' again (mean = 2.29 s). Thus the fading of contours is critical to the whole process.

How deep is the suppression of information within the scotoma? To investigate this we waited until the square had faded completely, then switched off the square and replaced it with an identical square that was shifted by about 0.4°. To our surprise, we found that the second square seems to 'move' or 'arrive' at its location, instead of just appearing out of nowhere. Even though the first square was not consciously visible it could nevertheless provide an input to apparent motion perception. A more formal experiment was conducted on the four subjects using a smaller square (1°×1°). On each trial we waited until the subject reported complete fading, then we switched off the square and replaced it with a new square that was either to the left or to the right of the original. Left–right trials were presented in random order and the subject's task was to simply report the direction. Performance on this task was well above chance (75 out of 80 trials; four subjects, 20 trials each). Furthermore, it was clear from the subjects' phenomenological reports that they were actually experiencing a sensation of motion and not simply reporting a change of location. We postulate, therefore, that the fading of the square from consciousness occurs from area V_4, the dorsolateral area (DL) and other 'form' areas, but that information about its motion is processed separately in the middle temporal area (MT).

Our next experiment also explored the depth of suppression within the scotoma. We waited until the square was replaced with twinkle, then we suddenly introduced a small (0.2°) red test-probe in the centre of the grey square. All four subjects reported that the spot instantly 'overcame' the filling in, but only in the small region corresponding to the spot, so that they saw a red spot surrounded by the filled-in twinkle that did not

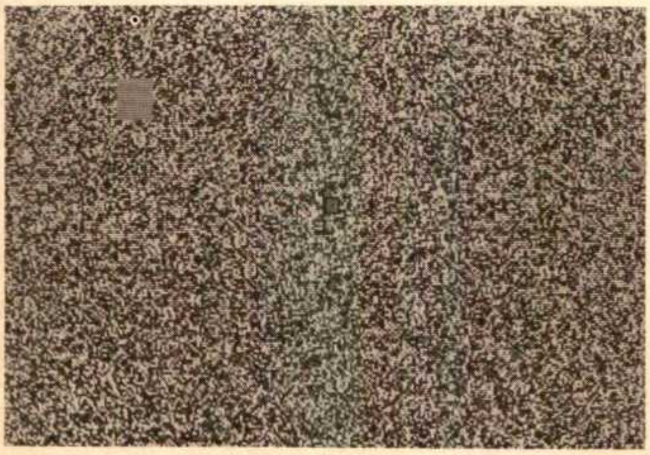


FIG. 1 Stimulus used to produce an artificial perceptual scotoma. The background consisted of twinkling spots of eight different grey levels. The square subtended 1.5°×1.5° and it had the same mean luminance (50 cd-m^{-2}) as the twinkling texture. The fixation spot was about 6° away from the border of the square. On steady fixation the square vanished in about 5 s and was filled in by the twinkling noise in the surround. A similar fading and filling in of texture was originally described by Ramachandran and Anstis[7] and Ramachandran[8] but in their stimulus the square was a 'window' filled with horizontally moving dots rather than a homogeneous grey. Also, we found that if the square was very small (<0.2°) it could be seen to vanish even if it was very bright or dark, that is non-equiluminous with the surround. (The effect could then be seen even if the fixation spot was only 2° from the square.) The fading occurred even more quickly (<2 s) if the square was in a different stereoscopic plane (nearer or further) than the twinkling texture. This was confirmed by all four subjects.

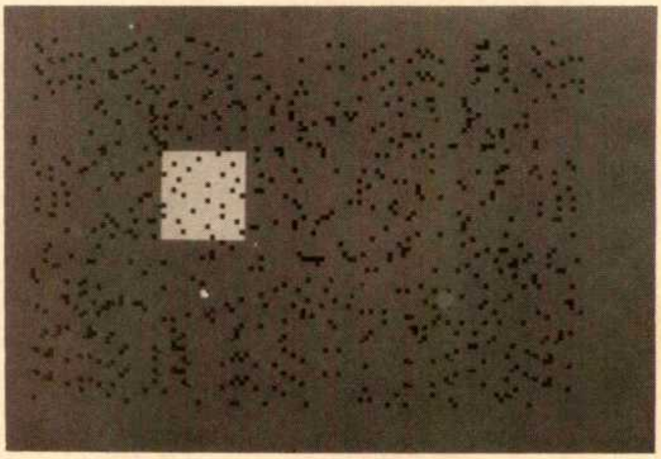


FIG. 2 Schematic illustration of stimulus used to demonstrate independent filling-in mechanisms for colour and twinkling texture. The homogeneous grey square subtended 2.6°×2.8°. The black spots in the square subtended 5 minutes of arc and moved horizontally as in a 'conveyor belt'. The surround consisted of randomly flickering ('twinkling') black spots on a homogeneous pink background (depicted dark grey) whose mean luminance was the same (80 cd m^{-2}) as the grey square. The background subtended 19°×15°. Four subjects were asked to view this display and asked whether the twinkle and colour both filled in at the same time, the twinkle filled in first, or the colour filled in first. On most trials the subjects reported that the colour filled in first (mean = 2.83 s; $n = 40$) followed a few seconds later by the twinkling noise (mean = 4.05 s).

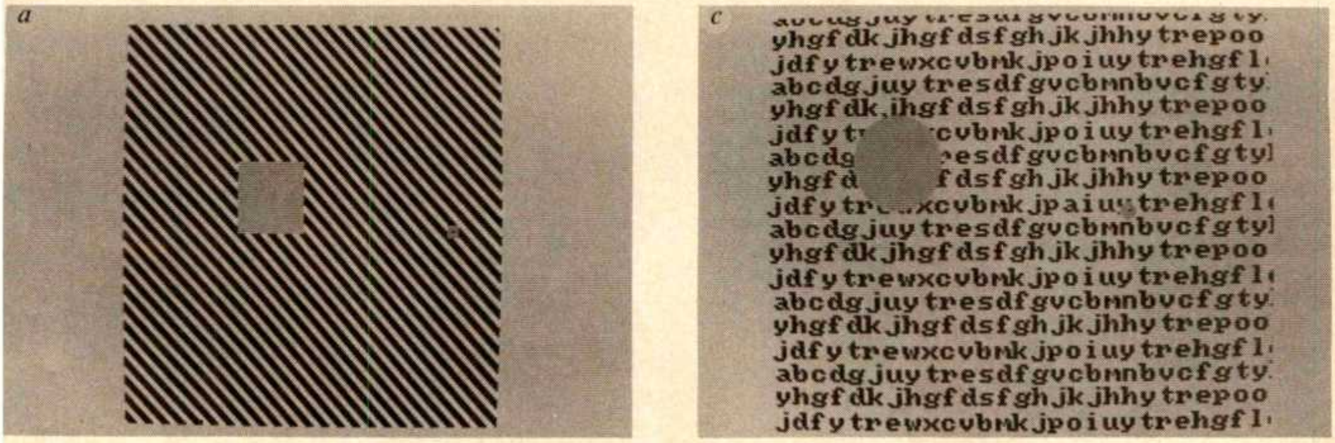
ctually exist around it on the retina. That the red spot is seen be actually surrounded by the filled-in twinkle is additional vidence for an active filling-in process. If no such process exists, ne might have simply experienced a vague inability to decide hat was immediately around the red spot.

Next, we explored the temporal characteristics of the filling-in rocess using Fig. 1. After filling in had occurred, we switched ff the entire display and replaced it with a homogeneous grey eld of equal luminance to the square (the square subtended .3° × 1.3°). We now found that there was a square patch of winkly noise in the area that was originally occupied by the quare, and this patch persisted for as long as 2–3 s (mean for our subjects = 2.44 s; $n = 40$). We conclude that a time-varying eural representation of the surrounding twinkle is created ithin the scotoma and perhaps this representation persists for –3 s after the display is switched off. An alternative possibility that the twinkle creates some peculiar state of adaptation in he surround, and this adaptation subsequently 'induces' a patially heterogeneous twinkle in the region corresponding to he square. Even so, whatever mechanism is responsible for this nduction of twinkle in the region originally occupied by the quare is unlikely to be very different from the process causing he filling in of the scotoma in the first place. What is truly emarkable about the percept is its dynamic nature, that it is ctually seen to twinkle within the scotoma. When we changed he temporal frequency of the surrounding twinkle to about 0 Hz, from 30 Hz, we found that the perceived temporal requency of the persistent twinkling patch was also reduced orrespondingly.

Is the filling-in process specific to the pattern in the surround? Ve tried to answer this by using displays such as Fig. 3*a*, in hich the surround was a diagonal twinkling ('counterphase flickering') square wave grating. We waited for fill to occur, then replaced the background lines with twinkling two-dimensional noise. Subjects typically reported that this procedure restored the grey square momentarily (1 s) and that it had to be filled in again by the new twinkling texture in the surround. Scotomas can also be produced, although less vividly, using stationary patterns such as Fig. 3*b* and *c*. In Fig. 3*b* the surround consists of a stationary horizontal square wave grating, and in Fig. 3*c* it was composed of ordinary type-written English, Latin, or 'nonsense' text. The filling in of text was especially striking and was reported by all four subjects, although, needless to say, none of them could actually read the text within the filled-in region.

Equiluminous chromatic borders[13,14] and luminance edges[15] also tend to fade during optical image stabilization or steady fixation[12]. We found that this was true even if an achromatic (grey) square was displayed on an equiluminous yellow background. The square (subtending 2°) faded in about 3–4 s and was replaced by the yellow from the background. The presence of the yellow within the filled-in region cannot be explained in terms of conventional colour contrast or adaptation effects. Again, if a black spot (0.3° diameter) was introduced in the centre of the square, the filling in of yellow was observed right up to, but not beyond, the edges of the black spot. Yet, if we had a thin black ring (0.3° outer diameter) instead of a spot, the yellow filled the interior of the ring as well, its spread was not 'blocked' by the ring. So the word 'paint' might be a more appropriate metaphor than 'filling in'.

Our next experiment demonstrates an intriguing interaction between illusory contours[16–22] and the filling-in mechanism. The display consisted of a circular grey disk that straddled the vertical border between two equiluminous regions of different colour (pink and yellow). A vertical illusory contour was then


a
c
yhgf dk jhgf dsf gh jk jhhy trepoo
jdf y trewxcvbmk jpoiuy trehgf l
abcdg juy tresdf gvcbmnbvcf gty
yhgf dk jhgf dsf gh jk jhhy trepoo
jdf y t cvbmk jpoiuy trehgf l
abcdg esdf gvcbmnbvcf gty
yhgf d f dsf gh jk jhhy trepoo
jdf y tr xcvbmk jpaiu trehgf l
abcdg juy tresdf gvcbmnbvcf gty
yhgf dk jhgf dsf gh jk jhhy trepoo
jdf y trewxcvbmk jpoiuy trehgf l
abcdg juy tresdf gvcbmnbvcf gty
yhgf dk jhgf dsf gh jk jhhy trepoo
jdf y trewxcvbmk jpoiuy trehgf l
abcdg juy tresdf gvcbmnbvcf gty
yhgf dk jhgf dsf gh jk jhhy trepoo
jdf y trewxcvbmk jpoiuy trehgf l

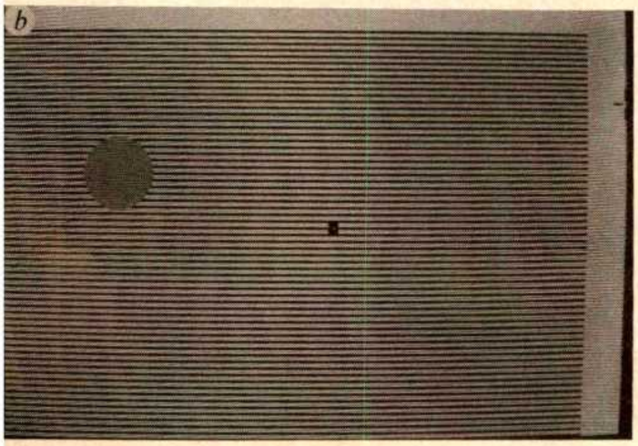


FIG. 3*a*, In this display the surround consisted of a counterphase flickering grating instead of twinkling noise. Fading and filling in took longer but could still be seen. *b*, In this display we have stationary continuously visible horizontal lines instead of a counterphase flickering grating. Fading and filling in can be seen after 15–20 s of steady eccentric fixation. We find that one out of every five or six subjects cannot achieve the steady fixation required for this effect. Also, on some trials the entire pattern fades (as in 'Troxler fading'[30]) instead of the square, an effect that is rarely seen in Fig. 1. *c*, The square is displayed against a 'nonsense' matrix of English letters. Again, the square fades and gets filled in by the alphabets, but subjects could not actually read the letters in the filled-in region.

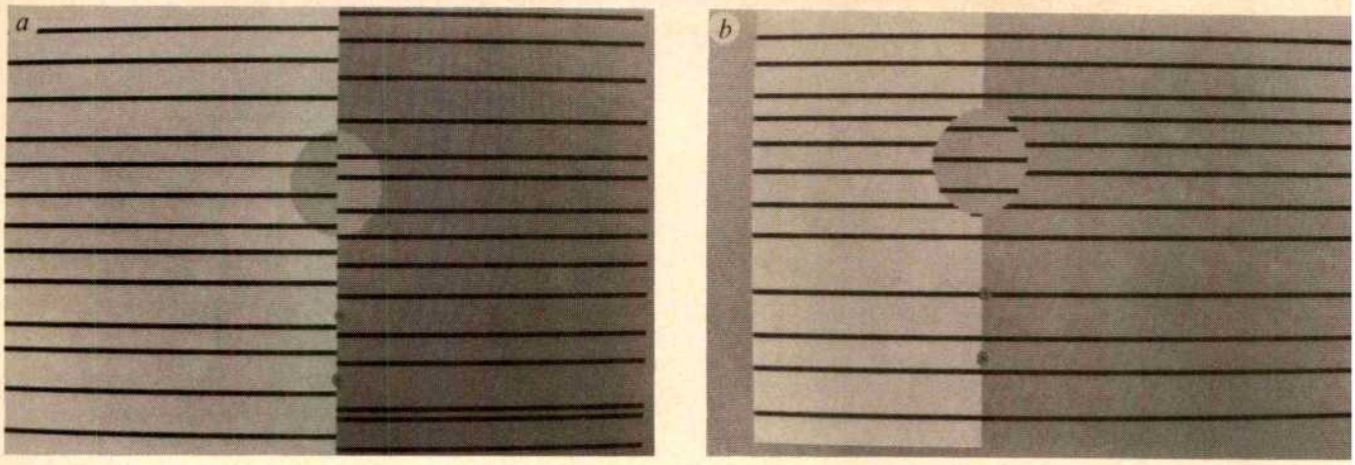


FIG. 4 Stimulus configuration used to demonstrate the effect of illusory contours on the filling-in process. *a*, the background was pink on the left (depicted light grey here) and green (depicted dark grey here) on the right and the circular disk was an equiluminous grey. Filling in occurred from both sides and formed a 'border' between the two filled-in colours that coincided with the illusory contour. In the absence of the central vertical illusor contour (*b*) the border appeared indistinct and unstable. The filling in of th disk also took longer in *b*, perhaps because of the illusory contours associated with the chromatic borders. Eleven naive subjects reported this effec filling in took place earlier in *a* than in *b* in 195 out of 220 trials.

introduced that coincided with the chromatic border and also continued across the centre of the grey disk (Fig. 4*a*). On steady eccentric fixation, subjects now reported that the colours filled in the disks from the two sides to form an illusory colour border corresponding to the illusory contour. If the illusory contour was not present the colour border within the scotoma usually appeared 'washed out', indistinct and unstable. (If the illusory contours coincided with the borders of the disk itself (Fig. 4*b*) the fading or filling-in processes were delayed.) We may conclude from this that the filling-in process is strongly influenced by early image segmentation, produced by illusory contours.

Finally, we wondered whether the filling in of the natural blind spot is also an active process that involves spatial integration. To explore this we used a 10°-wide red annulus (ring) whose inner margins just overlapped the outer margins of the blind spot, viewed monocularly. The red colour filled in the blind spot so that the stimulus looked like a homogeneous red disk rather than a ring. Yet when we introduced several concentric thin red rings around the annulus the blind spot appeared filled with rings instead of a homogeneous red, an effect that was confirmed by four naive subjects. Thus what fills in the blind spot is not merely what is immediately around it but als the pattern from the remote surround.

Considerable progress has recently been made in understanding the nature of neural representation underlying perception[23-29]. Our results suggest that the rules used by the visua system to fill in perceptual scotomas may help to elucidat further some of these processes. One intriguing possibility i that filling in simply reflects the activity of higher extrastriat neurons whose large receptive fields may straddle the boundarie of the grey square. Once the neurons that signal the square' border become fatigued, the signals from the surround may ge misattributed to the region corresponding to the scotoma. A second possibility is that feedback pathways[11,24] from extrastriate areas are used to create a representation of the surround i the portion of area 17 that corresponds to the scotoma. Thi theory complements, rather than negates, Sir David Brewster' hypothesis[6] that the filling in is achieved by 'the Divine Artificer' and it has the additional merit of being more easily testable.

Note added in proof. In patients with small (1°) retinal scotoma we find that filling in occurs for homogeneous colours an fine-grained static noise but not for dynamic twinkling nois (Fig. 1). □

Received 27 December 1990; accepted 27 February 1991.

1. Bender, M. B. & Teuber, H. L. *Arch. Neur. Psychiat.* **55,** 627-658 (1946).
2. Sergent, J. *Brain* **3,** 347-373 (1988).
3. Weiszcrantz, I. L. in *Current Problems in Animal Behavior* (eds W. H. Thorpe and O. Zangwill). 30-58 (Cambridge University Press, Cambridge, 1961).
4. Poppel, E. *Nature* **320,** 523-525 (1986).
5. Teuber, H. L. in *Brain and Conscious Experience* (ed. J. C. Eccles) 182-216 (Springer, New York, 1966).
6. Brewster, D. *Letters on Natural Magic*, addressed to Sir Walter Scott, Bart. (John Murray, London, 1832).
7. Ramachandran, V. S. & Anstis, S. M. 11th European Conference on Visual Perception. Bristol, UK, 1987.
8. Ramachandran, V. S. *Perception* **19,** 273 (1990); *The Artful Brain* (Oxford University Press, in the press).
9. Zeki, S. M. *Nature* **335,** 311-317 (1988).
10. Allman, J., Meizin, F. & McGuinnes, E. L. *Perception* **14,** 105-126 (1985).
11. Van Essen, D. C. *A. Rev. Neurosci.* **2,** 227-263 (1979).
12. Livingstone, M. S. & Hubel, D. H. *J. Neurosci.* **7,** 3416-3468 (1987).
13. Krauskopf, J. *Am. J. Psychol.* **80,** 632-637 (1961).
14. Crane, H. D. & Piantanida, T. P. *Science* 1078-1079 (1983).
15. Yarbus, A. L. *Eye Movement and Vision* (Engl. Trans. Haigh, B.; ed. Riggs, L. A.) (Plenum, New York, 1967).
16. Kaniza, G. *Organization in Vision* (Prager, New York, 1979).
17. Gregory, R. L. *Nature* **238,** 51-52 (1972).
18. Von Der Heydt, R. E., Peterhans, E. & Baumgartner, G. *Science* **224,** 1260-1262 (1978).
19. Ramachandran, V. S. *Perception* **14,** 127-134 (1985).
20. Ramachandran, V. S. *Soc. Neurosci. Abstr.* **15,** 624.
21. Ramachandran, V. S. *Perception and Psychophysics* **39,** 361-373 (1986).
22. Ramachandran, V. S. *Nature* **328**, 645-647 (1987).
23. Ramachandran, V. S. in *AI and the Eye* (eds Blake, A. & Troscianko, T.) 21-77 (Wiley, Bristol, 1990).
24. Finkel, L. H. & Edelman, G. M. *J. Neurosci.* **9,** 3188-3208 (1989).
25. Grossberg, S. & Mingolla, E. *Psychol. Rev.* **92,** 173-211 (1985).
26. Shepard, R. in *Perceptual Organization* (eds Kubovy, M. & Pomerantz, J. R.) 279-343 (Erlbaum, New Jersey, USA, 1981).
27. Julesz, B. *Foundations of Cyclopean Perception*, (University of Chicago Press, Chicago, 1971).
28. Poggio, T., Torre, V. & Koch, C. *Nature* **138,** 645-647 (1985).
29. Rock, I. *The Logic of Perception* (MIT, Cambridge, Massachusetts, 1983).
30. Troxler, D. in *Opthalmologische Bibliothek.* **II, PT2,** 1-53 (1804).

ACKNOWLEDGEMENTS. We thank W. Aiken, A. Saluda, D. Rogers-Ramachandran, F. Crick, T. Sejnowsky, P. Churchland and C. Ramachandran for stimulating discussions, and the Airforce Office of Scientific Research for support. R.L.G. was a McDonnel-Pew visiting scholar at UCSD.

Peptide selection by MHC class I molecules

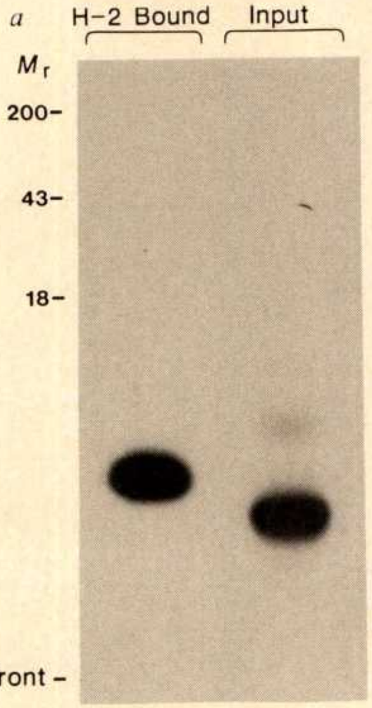
Ton N. M. Schumacher*, Marloes L. H. De Bruijn†, Leen N. Vernie*, W. Martin Kast†, Cornelis J. M. Melief†, Jacques J. Neefjes* & Hidde L. Ploegh*

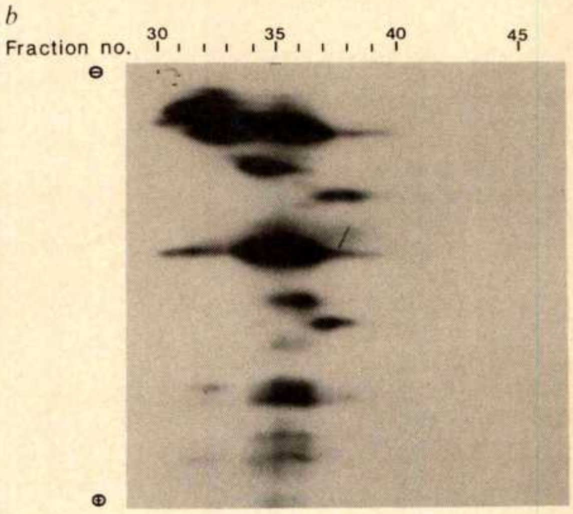
* Departments of *Cellular Biochemistry and †Immunology, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX, Amsterdam, The Netherlands

SYNTHETIC peptides have been used to sensitize target cells and thereby screen for epitopes recognized by T cells[1–7]. Most epitopes of cytotoxic T lymphocytes can be mimicked by synthetic peptides of 12–15 amino acids[8]. Although in specific cases, truncations of peptides improves sensitization of target cells[8,9], no optimum length for binding to major histocompatibility complex (MHC) class I molecules has been defined. We have now analysed synthetic peptide captured by empty MHC class I molecules of the mutant cell line RMA-S. We found that class I molecules preferentially bound short peptides (nine amino acids) and selectively bound these peptides even when they were a minor component in a mixture of longer peptides. These results may help to explain the difference in size restriction of T-cell epitopes between experiments with synthetic peptides and those with naturally processed peptides[10–12].

MHC class I molecules in the murine cell line RMA-S are unable to engage endogenous peptides[13–16]. Empty class I molecules formed in this cell line are unstable at 37 °C, but are expressed in considerable quantities at the surface at reduced temperatures (optimum, 26 °C; notation for this cell line at 26 °C, RMA-S(26)) and can be used to measure binding of peptide to class I molecules[14,15].

A peptide preparation derived from Sendai virus nucleoprotein and identified as a cytotoxic T-lymphocyte (CTL) epitope (SV12 peptide; residues 321–332 of the nucleoprotein: HGEFAPGNYPAL in the single-letter amino-acid code) binds and stabilizes empty H-2 K^b and D^b molecules[17]. A 16-residue Sendai peptide extended at its carboxy terminus (SV16 peptide; residues 321–336; sequences in Fig. 1*c*) could also stabilize empty class I molecules and sensitize targets for lysis by CTL. SV12 was radiolabelled by iodination, presented to RMA-S(26) cells, and class I-associated peptide was analysed on an SDS–tricine gel system[18]. The class I-bound material did not comigrate





c

SV12	NH_2-H G E F A P G N **Y** P A L-amide
SV16	NH_2-H G E F A P G N **Y** P A L W S **Y** A-amide
SV9	NH_2-F A P G N **Y** P A L-COOH

FIG. 1 *a*, MHC class I-bound peptide is not identical to the input peptide. RMA-S cells were cultured for 48 h at 26 °C, washed and incubated for 1 h with 5 μM ^{125}I-labelled Sendai nucleoprotein peptide (residues 321–332, SV12 peptide) at 26 °C. After washing and lysis, class I molecules were isolated by immunoprecipitation and analysed by electrophoresis on a SDS–tricine gel. Relative molecular mass markers are indicated on the left (M_r). For small peptides, separation is based both on charge and SDS-binding properties, rather than the true molecular mass of the peptide. Note that the peptide bound to H-2 molecules does not comigrate with the major peptide in the input material. *b*, Heterogeneity of SV12 as revealed by two-dimensional HPLC and isoelectric focusing analysis. SV12 peptide was labelled by iodination and separated in the first dimension by reversed-phase HPLC. HPLC fractions were lyophilized and resuspended in RPMI. Small aliquots of all fractions were analysed by isoelectric focusing[20]. Only the region of the gel containing the majority of the labelled peptide is shown. Note that overexposure of one-dimensional isoelectric-focusing analysis of the different HPLC fractions reveals the presence of minor contaminants that elute in the vicinity of the main peak. *c*, Sendai nucleoprotein-derived peptides that can sensitize target cells for lysis by H-2K^b-restricted CTLs. Sequences are given in single-letter code. V8 protease recognition sites are underlined. Preferred sites of iodination catalysed by chloramine T (Y) are in bold. Amino-acid composition was determined on hydrolysed SV12 peptide. Yield of the separate amino acids relative to the most C-terminal amino acid (leucine) is given. Expected values are indicated between brackets (moles per mole of peptide). His, 0.7 (1); Gly, 1.6 (2); Glu 1.0 (1); Phe, 1.0 (1); Ala, 2.0 (2); Pro, 1.7 (2); Asp 0.7 (1); Tyr, 0.8 (1); Leu, 1.0 (1). Sequence analysis performed on an ABS gas phase sequenator shows that the peptide is of the expected sequence. The synthesis strategy probably does not result in peptides with internal deletions, because growing peptide chains that fail to couple are terminated with a blocking agent and cannot engage in further chain elongation. Truncations arise as a consequence of premature termination in the course of synthesis (N-terminal truncations), or partial hydrolysis during cleavage of peptide of the resin (N- or C-terminal truncations). Further possible contaminants result from incomplete deprotection and deamidation.

METHODS. Peptides were synthesized by standard t-boc chemistry on a Biosearch SAM 2 peptide synthesizer, dissolved in PBS medium and stored at 4 °C. Peptides were iodinated in a reaction catalysed by chloramine T (ref. 21) to a specific activity of about 50 Ci mmol^{-1}. The mouse lymphoma cell lines transformed with Rauscher virus, RMA and RMA-S (refs 22, 23), were maintained in DMEM medium supplemented with 10% FCS and antibiotics. Before use, cells were cultured for 48 h in DMEM–10% FCS supplemented with 20 mM HEPES buffer and antibiotics (10^6 cells per ml) in a waterbath at 26 °C. Cells were then incubated for 1 h with 5 μM ^{125}I-labelled Sendai nucleoprotein peptide at 26 °C. Cells were washed three times with PBS and lysed on ice in TX-100 lysis buffer. Class I molecules were immunoprecipitated and analysed by electrophoresis on SDS–tricine gel[18]. Gels were then frozen and exposed to Kodak XAR-5 films at −70 °C. Peptide binding assays were performed in the absence of FCS to avoid peptide modifications by serum components[7]. *b*, Radiolabelled SV12 was resolved by HPLC on a Beckman ODS C-18 RP 4.6 × 250-mm reversed-phase column. After initial isocratic elution in 0.1% TFA for 2 min, linear gradient elution to 0.1% TFA–50% acetonitrile was done for 50 min. Fractions (1 ml) were collected, lyophilized and run on one-dimensional isoelectric focusing gels[24].

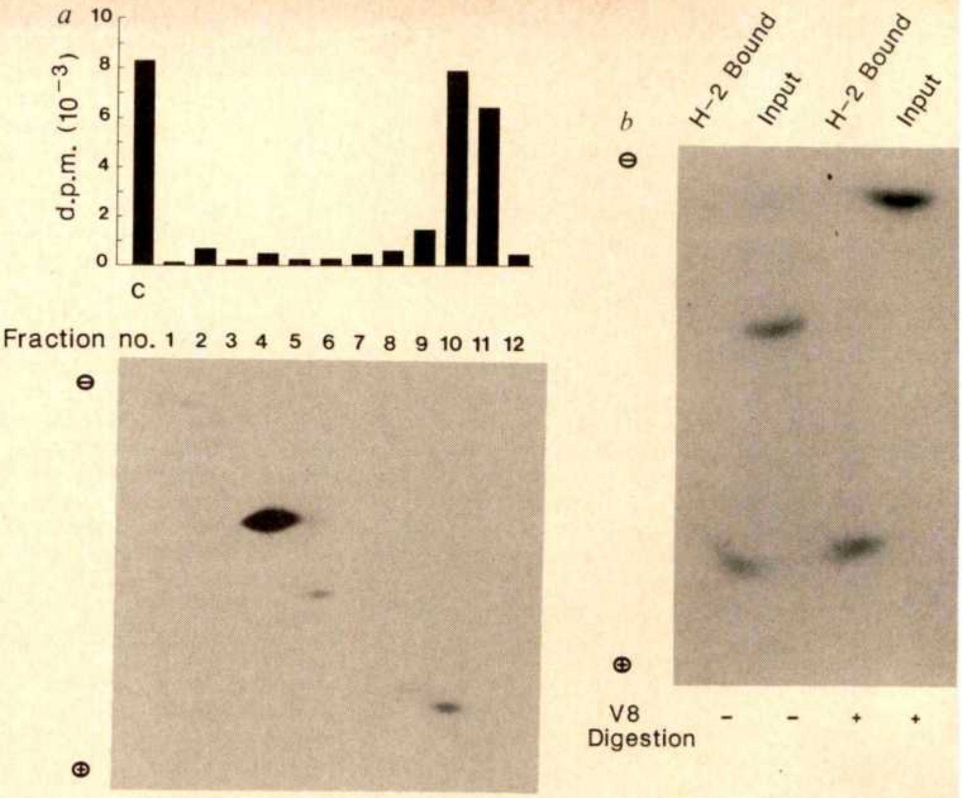


FIG. 2 *a*, Class I binding peptide can be isolated by preparative isoelectric focusing. SV12 peptide was radiolabelled by iodination and subsequently separated by preparative one-dimensional isoelectric focusing done as described[24], but without urea in the gel. After isoelectric focusing, peptide was eluted from 1-cm gel slices, lyophilized and resuspended in RPMI. Small aliquots of the different fractions were analysed by rerun on one-dimensional isoelectric focusing (bottom). The fractions were used in a peptide-binding assay (top), as described in Fig. 1. RMA-S(26) cells were incubated with a 1:10 dilution in DMEM-Hepes of fractions 1–12. After washing and lysis of the cells, class I antigens were immunoprecipitated, and the presence of class I-associated peptide was determined by gamma-spectrometry. The binding of control, unfractionated ^{125}I-labelled SV12 peptide to class I molecules is shown on the left of the figure. *b*, V8 protease insensitivity of class I captured peptide. RMA-S(26) cells were incubated with ^{125}I-labelled SV12 peptide as described in Fig. 1. Class I-associated peptide was isolated by immunoprecipitation of class I antigens. Equal amounts of d.p.m. of the class I-associated peptide and the input SV12 peptide were incubated for 5 min at 95 °C in 200 mM Tris, 200 mM tricine, 0.2% SDS pH 8.25, cooled on ice and subsequently incubated for 30 min at 37 °C with or without 0.5 mg per ml V8 protease (Sigma), which cleaves the C terminal from glutamic acid. Peptides were then analysed by one-dimensional isoelectric focusing[24] as described, but without urea.

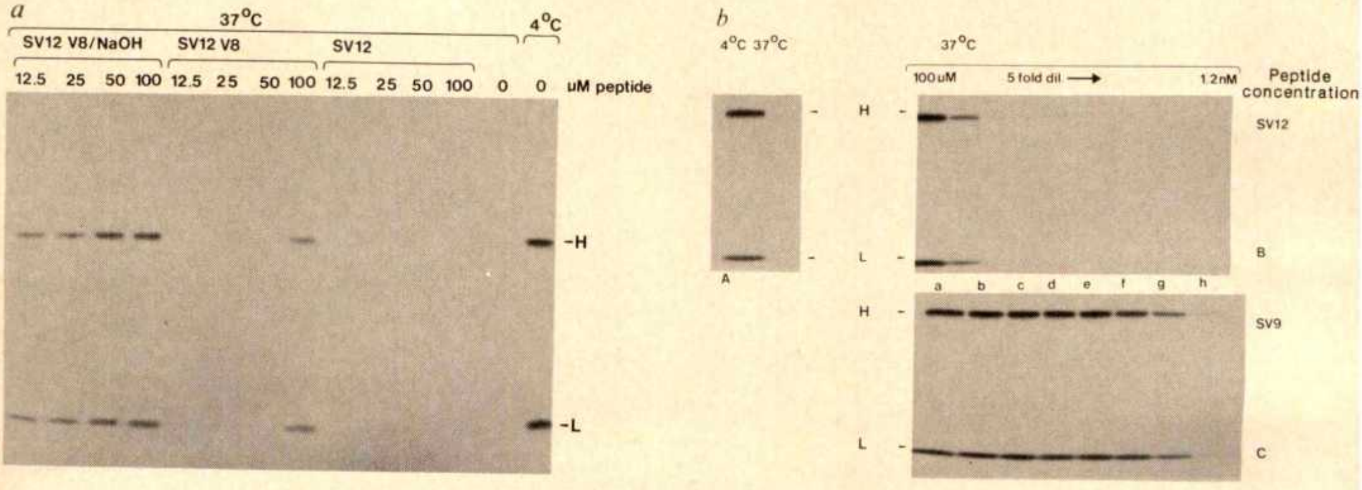
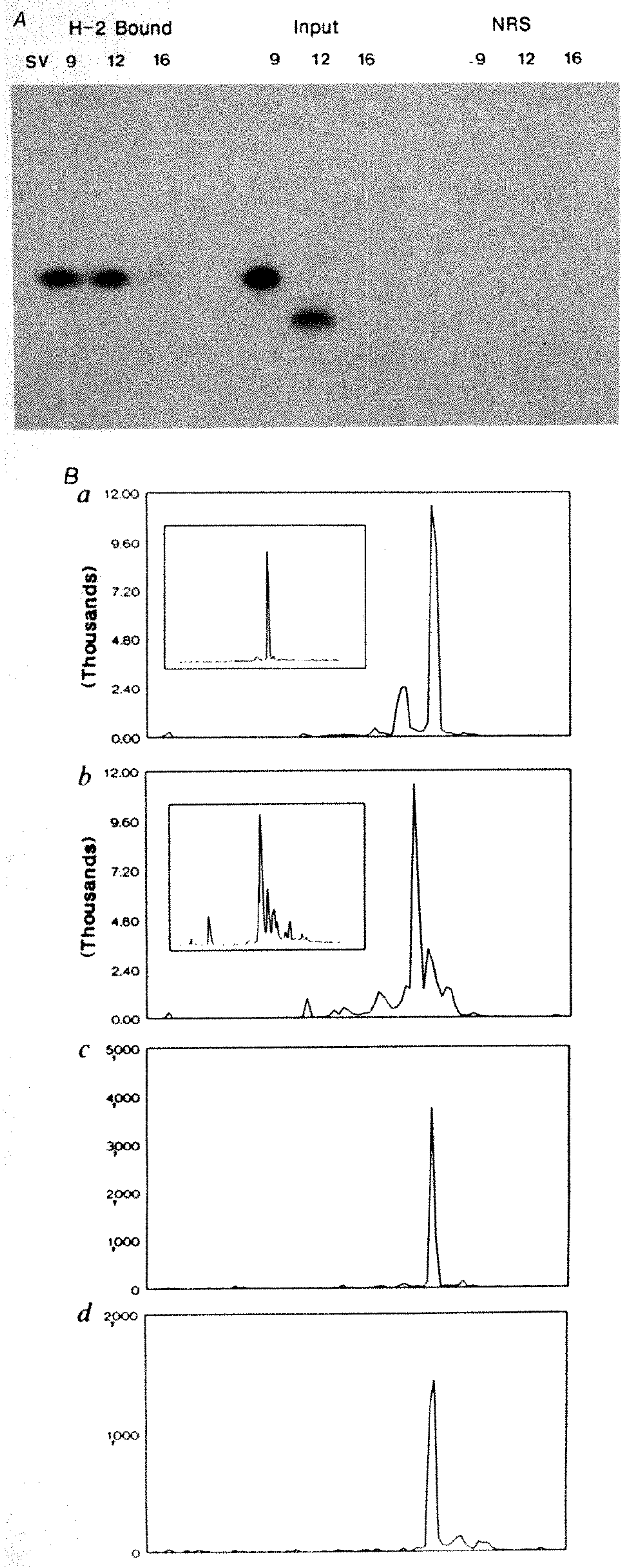


FIG. 3 *a*, A combined V8–NaOH treatment of the SV12 peptide improves the ability to stabilize empty class I molecules. The SV12 peptide was digested with V8 protease or mock digested as described in Fig. 2*b*. Subsequently, peptides were either diluted 20-fold in 50 mM NaOH, or 40-fold in 25 mM NaOH, 500 mM Tris, pH 7.8, and incubated at 95 °C for 30 min. Peptides diluted in 50 mM NaOH were diluted twofold in 1 M Tris, pH 7.8, afterwards. One-dimensional isoelectric focusing confirmed that incubation at 95 °C at neutral pH does not lead to deamidation, whereas incubation in 50 mM NaOH leads to considerable deamidation. Mock-treated, V8-treated and V8–NaOH-treated SV12 peptides were used in a class I stabilization assay. RMA–S(26) cells were cell-surface labelled by lactoperoxidase catalysed iodination[20]. After washing and lysis, cell lysates were incubated with the different peptides for 1 h at the concentrations indicated. Lysates were then exposed for 1 h to 4 °C or 37 °C, class I antigens were isolated using an anti H-2^{b} serum and immunoprecipitates were analysed on a 12% SDS–PAGE gel. The V8–NaOH-treated SV12 peptide stabilizes class I molecules at much lower concentrations than the original SV12 peptide preparation. *b*, Stabilization of empty class I molecules by SV12 and SV9 peptides. RMA–S(26) cells were cell-surface labelled by lactoperoxidase-catalysed iodination. After washing and lysis, cell lysates were incubated with the different peptides for 1 h at the concentrations indicated in Methods. Lysates were then exposed for 1 h to 4 °C or 37 °C. Class I antigens were isolated using an anti H-2^{b} serum and immunoprecipitates were analysed on a 12% SDS–PAGE gel. Note that the SV9 peptide can stabilize class I molecules when present at concentrations as low as 6 nM, whereas the SV12 peptide is only active at concentrations >20 μM. On the assumption that only SV9 is capable of stabilization this would imply that the amount of contaminating 'SV9 peptide' in this preparation is less than 0.1%.

METHODS. Cells were cell-surface labelled at 0 °C using lactoperoxidase-catalysed iodination as described[24], washed thrice with PBS and lysed on ice in TX-100 lysis buffer (10 mM Tris, pH 7.8, 140 mM NaCl, 1% Triton X-100, 1 mM PMSF, 1 μg ml^{-1} trypsin inhibitor, 30 mTIU (trypsin inhibitory units) ml^{-1} aprotinin). Lysates were incubated with peptides for 1 h at 4 °C, in *a* at the concentrations indicated, and in *b* at the following concentrations: a, 100 μM; b, 20 μM; c, 4 μM; d, 800 nM; e, 160 nM; f, 32 nM; g, 6.4 nM; h, 1.2 nM. Lysates were then exposed for 1 h to either 4 °C or 37 °C. Class I antigens were immunoprecipitated using a rabbit serum raised against purified H-2^{b}.

with the major or any obvious minor peptide in the input material (Fig. 1*a*). A peptide of similar mobility was selected by RMA-S(26) from radiolabelled SV16 (Fig. 4A) Contaminating peptides are likely to include truncations (see legend to Fig. 1). For the two synthetic peptides containing the same core sequence, a single truncated peptide might therefore be able to associate with class I molecules. Alternatively, SV12 and SV16 could be trimmed by proteolysis to the same length after binding to class I molecules. To check for the presence of possible peptide contaminants, SV12 was radiolabelled, and separated by two-dimensional HPLC–isoelectric focusing. Several minor contaminants were present (Fig. 1*b*).



SV12 preparation was separated by isoelectric focusing (Fig. 2*a*, bottom), and the resulting fractions were used in peptide binding assays. No class I-binding peptide could be detected in the peak fraction (fraction 4), and only fractions 10 and 11, which contain a small part of the input material, contained class I-binding peptide (Fig. 2*a*, top). Class I-bound peptide and the material in fraction 10 were indistinguishable in size (tricine gel) and charge (isoelectric focusing: not shown). These results suggest that a proteolytic conversion, catalysed either by the class I molecules themselves, or by associated proteases, is not responsible for the generation of the peptide. The material in isoelectric-focusing fraction 10 and the class I-eluted material were subjected to Edman degradation. In both cases the label was recovered after six cycles of degradation, suggesting that the three N-terminal residues of SV12 were absent from this peptide. Indeed, the class I-captured peptide was insensitive to treatment with V8 protease (Fig. 2*b*).

The removal of the first three residues of SV12 results in a peptide with a higher isoelectric point than that of the H-2-binding peptide. The C-terminal leucine-amide is therefore probably lost or modified. Because this leucine can be replaced by only a few amino acids in SV16 and still result in target-cell sensitization (W.M.K. *et al.*, unpublished observation), deletion seems unlikely.

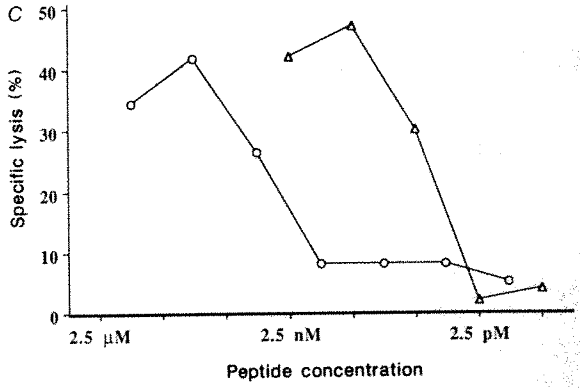


FIG. 4 *A*, Analysis of input and MHC-captured SV peptides on SDS–tricine gel. SV9, SV12, and SV16 peptides were labelled by cloramine T-catalysed iodination and offered to RMA-S(26) cells as described in the legend to Fig. 1. MHC captured and input peptides were analysed on SDS–tricine gel. H-2: H-2 captured peptide; Input: input peptide; NRS: normal rabbit serum control. Note comigration of MHC-bound peptides for SV9, SV12 and SV16. *B*, HPLC analysis of input and MHC-captured SV peptides. *a*, Unlabelled (inset) and labelled SV12 peptide; *b*, unlabelled (inset) and labelled SV9 peptide; *c*, MHC-captured SV12 peptide; *d*, MHC-captured SV9 peptide. *C*, Target cell sensitization by SV16 and SV9 peptides. SV16 (○) and SV9 (△) peptides were tested at different concentrations for sensitization of target cells to lysis by a Sendai virus-specific CTL clone[17,26]. Half-optimum specific ^{51}Cr release was reached at a concentration of 10 nM SV16 and 20 pM SV9 peptide.

METHODS. *B*, H-2-captured and input-radiolabelled peptides were prepared as described in the legend to Fig. 1. Samples were dissolved in 0.05% TFA and analysed by reverse phase HPLC on a C18 reverse phase HPLC column (Millipore Waters). Buffers: A, H_2O–0.05% TFA; B, acetonitrile–0.05% TFA. Elution profile 0–3 min 95→95% A, 3–5 min 95→85% A, 5–23 min 85→60% A, 23–34 min 60→35% A. Fractions (0.3 min) were collected and radioactivity in the individual fractions was determined by gamma-spectrometry. The peak of radioactivity was in fraction 61 for *b*, and in fractions 64–65 for *a*, *c* and *d*. Material captured from SV16 likewise eluted at fractions 64–65 (not shown). *C*, B6 mouse embryo cells were cultured for 2 days in the presence of 50 U per ml recombinant interferon-τ to increase class I expression. Cloned CTL (2×10^4) were added to 2×10^3 ^{51}Cr-labelled B6 MEC in a final volume of 200 μl in 96-well U-bottomed plates. Cells were incubated for 6 h at 37 °C in humidified air with 5% CO_2. ^{51}Cr release was calculated as described[26]. Peptides were present during the whole assay at the concentrations indicated.

Empty class I molecules in detergent lysates rapidly change their conformation at 37 °C, which can be prevented by incubation with presentable peptides[15]. SV12 digested with V8 protease, and V8-digested SV12 partially deamidated by dilute NaOH, were tested for their ability to stabilize empty class I molecules. The combination of V8 digestion and deamidation yields a peptide preparation much more potent at stabilizing empty class I molecules (Fig. 3*a*). SV9 peptide with a free carboxyl group binds to and stabilizes class I molecules in detergent lysates at concentrations as low as 6 nM (>4,000-fold lower concentration than required for stabilization by the SV12 peptide; Fig. 3*b*). If a peptide corresponding to SV9 were solely responsible for the stabilizing effect of SV12, then it probably constitutes less than 0.1 per cent of total peptide. The material captured from SV9, SV12 and SV16 comigrates with input SV9 on SDS-tricine gel (Fig. 4*A*), one-dimensional isolectric focusing (not shown) and HPLC (shown for SV12 in Fig. 4*B*) under conditions where SV9, SV12 and SV16 are resolved. The ability of the different Sendai virus peptides to sensitize target cells for lysis by Sendai-specific CTL shows that SV9 can induce sensitization at 100-1,000-fold lower concentrations than the longer peptides derived from Sendai virus (Fig. 4C). Concentrations of the SV9 peptide in the picomolar range are sufficient to sensitize for lysis by CTL.

Both ends of the groove in HLA-A2 and A68 seem to be closed[19] and could therefore put an upper limit on the size of binding peptides. Peptides longer than nine amino acids might not fit into the groove as tightly as those of optimal size (8 or 9 amino acids), but they could bind at one end of the groove, with one of their sides projecting away from it. Alternatively, both ends of the peptide could be neatly tucked in, the middle part interacting less tightly. A free terminal carboxyl group is apparently essential for high-affinity binding of the Sendai peptide to class I molecules. Therefore, the size limit for peptides might not only reflect the boundaries of the peptide-binding groove, but also the positioning of the free carboxyl group. That only few amino-acid substitutions are allowed for the SV9 core in SV16, whereas residues outside this core can be replaced by all other amino acids (W.M.K. *et al.*, unpublished observation), suggests that there might also be a lower limit to the size of class I binding peptides. The set of endogenous peptides extracted with trifluoroacetic acid from intact cells and capable of sensitizing for lysis by CTL is determined by the class I type of the donor cell[10], although direct selection of peptide by class I molecules themselves was not shown. These endogenously derived peptides are shorter and more potent than the T-cell epitopes deduced from experiments using synthetic peptides, and are similar in size to the Sendai peptide. Endogenous peptides directly isolated from class I molecules in cells infected with vesicular stomatitis virus were also remarkably homogeneous and composed of only eight residues[11]. This homogeneity has been suggested to be imposed by the peptide-generating or translocating machinery, or to be an intrinsic property of class I molecules[10-12]. Our experiments show that class I molecules themselves are remarkably selective in peptide binding. □

Received 13 December 1990; accepted 1 March 1991.

1. Townsend, A. R. M. *et al. Cell* **44,** 959-968 (1986).
2. Maryanski, J. L., Pala, P., Corradin, G., Jordan, B. R. & Cerottini, J. C. *Nature* **324,** 578-579 (1986).
3. Gotch, F., Rothbard, J., Howland, K., Townsend, A. & McMichael, A. *Nature* **326,** 881-882 (1987).
4. Kast, W. M. *et al. Cell* **59,** 603-614 (1989).
5. Oldstone, M. B. A., Whitton, J. L., Lewicki, H. & Tishon, A. *J. exp. Med.* **168,** 559-570 (1988).
6. Gotch, F., McMichael, A. & Rothbard, J. *J. exp. Med.* **186,** 2057 (1988).
7. Moore, M. W., Carbone, F. R. & Bevan, M. J. *Cell* **54,** 777-785 (1988).
8. Townsend, A. & Bodmer, H. *A. Rev. Immun.* **7,** 601-624 (1989).
9. Reddehase, J. M., Rothbard, J. B. & Koszinowski, U. H. *Nature* **337,** 651-653 (1989).
10. Rötzschke, O. *et al. Nature* **348,** 252-254 (1990).
11. Van Bleek, G. M. & Nathenson, S. G. *Nature* **348,** 213-216 (1990).
12. Elliot, T., Townsend, A. & Cerundolo, V. *Nature* **348,** 195-197 (1990).
13. Townsend, A. *et al. Nature* **340,** 443-448 (1989).
14. Ljunggren, H. G. *et al. Nature* **346,** 476-480 (1990).
15. Schumacher, T. N. M. *et al. Cell* **62,** 563-567 (1990).
16. Townsend, A. *et al. Cell* **62,** 285-295 (1990).
17. Kast, W. M. *et al. Proc. natn. Acad. Sci. U.S.A.* (in the press).
18. Schägger, H. & von Jagow, G. *Analyt. Biochem.* **166,** 368-379 (1987).
19. Bjorkman, P. J. & Parham, P. *A. Rev. Biochem.* **59,** 253-288 (1990).
20. Phillips, D. R. & Morrison, M. *Biochem. biophys. Res. Commun.* **40,** 284-289 (1970).
21. Hunter, W. M. & Greenwood, F. C. *Nature* **194,** 495-496 (1962).
22. Ljunggren, H. G. & Kärre, K. *J. exp. Med.* **162,** 1745-1759 (1985).
23. Kärre, K., Ljunggren, H. G., Piontek, G. & Kiesling, R. *Nature* **319,** 675-678 (1986).
24. Neefjes, J. J., Breur-Vriesendorp, B. S., Van Seventer, G. A., Ivanyi, P. & Ploegh, H. L. *Hum. Immu* **16,** 169-181 (1986).
25. Hämmerling, G. J., Hämmerling, U. & Lemke, H. *Immunogenetics* **8,** 433-445 (1979).
26. Kast, W. M., Bronkhorst, A. M., de Waai, L. P. & Melief, C. J. M. *J. exp. Med.* **164,** 723-738 (1986

ACKNOWLEDGEMENTS. We thank E. Palmieri and S. Nathenson for the gift of rabbit anti H-2^b serum J. Nieland and R. Brandt for technical assistance, R. van der Valk, P. van Balen and T. Lutz fo assistance with HPLC and peptide iodinations, D. Wiley for helpful comments, and W. J. Weijer an H. J. Bak from Eurosequence, Groningen, for amino-acid and sequence analysis. This research wa supported by the Netherlands Cancer Foundation and the Netherlands Organisation for Scientifi Research.

Enhancement of T-cell activation by the CD43 molecule whose expression is defective in Wiskott–Aldrich syndrome

John K. Park[*], Yvonne J. Rosenstein[*†], Eileen Remold-O'Donnell[§||], Barbara E. Bierer[*‡], Fred S. Rosen[§||], & Steven J. Burakoff[*§]

[*] Division of Pediatric Oncology, Dana Farber Cancer Institute,
[‡] Division of Hematology, Brigham and Women's Hospital,
[||] The Center for Blood Research and the Departments of [§] Pediatrics and
[‡] Medicine, Harvard Medical School, Boston, Massachusetts 02115, USA

CD43 (sialophorin, leukosialin, leukocyte large sialoglycoprotein), a heavily sialylated molecule found on most leukocytes and platelets, was initially identified as a major glycoprotein of mouse, rat and human T cells[1-8]. CD43 expression is defective on the T cells of males with the Wiskott–Aldrich syndrome, an X chromosome-linked recessive immunodeficiency disorder[9]. Affected males are susceptible to opportunistic infections and do not respond to polysaccharide antigens, reflecting defects in cytotoxic and helper T-cell functions. Anti-CD43 monoclonal antibodies have a modest costimulatory effect on T cells, natural killer cells, B cells and monocytes[10-14], and one such antibody has been shown to activate T cells directly[15]. To investigate a possible physiological role for CD43, a complementary DNA encoding the human protein[16,17] was introduced into an antigen-responsive murine T-cell hybridoma[18]. We observed that CD43 enhances the antigen-specific activation of T cells and that the intracellular domain of CD43, which is hyperphosphorylated during T-cell activation[19-21], is required for this function. We also found that antigen-presenting cells can bind specifically to immobilized purified CD43 and that the binding can be inhibited by liposomes containing CD43 as well as by anti-CD43 monoclonal antibodies.

The murine T-cell hybridoma By155.16 produces interleukin-2 (IL-2) following stimulation of its T-cell receptor/CD3 complex (TCR-CD3) by anti-CD3 monoclonal antibody. The TCR of By155.16 is specific for HLA-DR antigens such as those expressed by Daudi, a Burkitt's lymphoma-derived B-cell line. As a result, By155.16 can be stimulated to produce IL-2 by coculture with Daudi. But the particular subclone of By155.16 studied here can only be stimulated to produce IL-2 if it has been previously transfected with genes encoding human T-cell accessory molecules such as CD4, which recognizes HLA-DR, or CD2, which recognizes LFA-3[18,22,23]. The dependence of the Daudi-induced activation of this subclone on such accessory molecules was exploited to determine whether CD43 could similarly function as an accessory or costimulatory molecule.

[†] Permanent address: Instituto de Investigaciones Biomedicas, UNAM, Mexico.

A cDNA encoding either the wild-type CD43 protein (CD43) or a CD43 protein with the intracellular domain deleted (ΔCD43) was inserted into the plasmid pFneo[24], which contains he G418 resistance gene (neo^r) (Fig. 1). By155.16 cells were ransfected by electroporation[25] with pFneo, pCD43Fneo, or pΔCD43Fneo, selected for resistance to G418, and subcloned by limiting dilution. From each transfection group, clones expressing equivalent levels of TCR-CD3 and/or the extracellular domain of CD43 where appropriate were selected for further study (Fig. 2*a* and *b*). Also selected were CD43$^-$, CD43$^+$ and ΔCD43$^+$ clones which were TCR-CD3$^-$. To assess the ability of the different clones to generate IL-2, they were stimulated with anti-CD3 monoclonal antibody. Those clones expressing TCR-CD3 responded equivalently (Fig. 3*a*), whereas those expressing equivalent levels of CD43 or ΔCD43 but no detectable TCR-CD3 produced <20 U IL-2. To determine whether CD43 could have a costimulatory role in T-cell activation induced by antigen-presenting cells, the clones were cocultured with Daudi. Of the TCR-CD3$^+$ clones, the CD43$^+$ clone responded to Daudi stimulation, but the CD43$^-$ and ΔCD43$^+$ clones did not (Fig. 3*a*). Likewise, none of the TCR-CD3$^-$ clones responded to Daudi stimulation (IL-2 production <20 units). In the presence of TCR-CD3, therefore, CD43 can serve as an accessory molecule in the activation of T cells by cellular antigens. The role of signal transduction was highlighted by the inability of the ΔCD43$^+$ clone to respond to Daudi stimulation although it responded well to stimulation by anti-CD3 monoclonal antibody.

To confirm that CD43 can serve as an accessory molecule, we examined a series of 48 CD43$^-$, 48 CD43$^+$, and 48 ΔCD43$^+$ By155.16-derived uncloned TCR-CD3$^+$ cell lines. As shown in Fig. 3*b*, 19 of the CD43$^+$ lines (40%), but none of the CD43$^-$ and ΔCD43$^+$ lines responded to Daudi stimulation. Statistical analysis of the three groups revealed that the mean IL-2 production induced by Daudi stimulation of the 48 CD43$^-$ lines did not differ significantly from that of the 48 ΔCD43$^+$ lines. The mean of the 48 CD43$^+$ lines was, however, significantly greater than that of the other groups ($P<0.001$, by Student's t-test). This supports the conclusion that in the presence of TCR-CD3, CD43 can increase the efficacy of T-cell activation by antigen-presenting cells.

Binding assays were used to examine the specificity of the CD43-mediated interaction between T cell and antigen-presenting cell. Immunoaffinity-purified CD43 molecules were adsorbed to 96-well plastic tissue culture plates and the ability of Daudi cells and control A20 murine B cells to adhere to the CD43-coated plates was assessed. A significantly higher percentage of Daudi cells than A20 cells adhered to the CD43-coated plates (Fig. 4*a*). Preincubation of Daudi cells with liposomes containing purified CD43 molecules abrogated this adhesion,

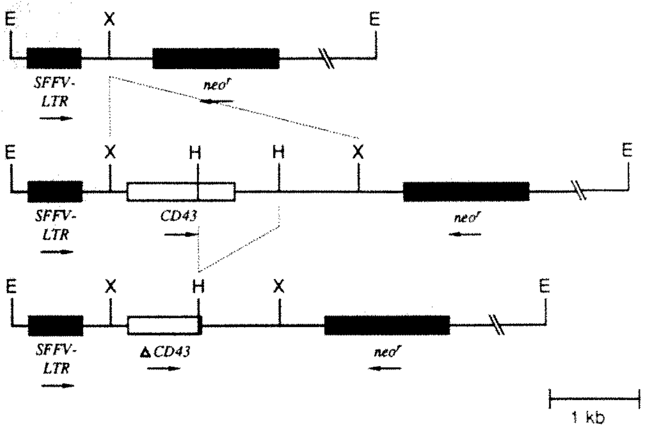


FIG. 1 Plasmid constructs. A full length CD43 cDNA clone (gift of C. S. Shelley and Michael Carroll) was subcloned into the pFneo plasmid (top; gift of R. Germain) to generate the pCD43Fneo plasmid (middle). Plasmid pCD43Fneo was in turn modified to generate the pΔCD43Fneo plasmid (bottom). The dotted lines between the three plasmids indicate areas of insertions and deletions. Plasmid pFneo and its derivatives contain the G418 resistance gene neo^r and the spleen focus-forming virus long terminal repeat (SFFV-LTR) which promotes the expression of 5' → 3'-oriented genes located near its 3' terminus. Plasmid pCD43Fneo-transfected cells can express the full-length (381 amino acids) form of CD43, and pΔCD43Fneo-transfected cells a truncated form of CD43 retaining all the 259 predicted extracellular and transmembrane domain amino acids, but only the first of the 123 predicted intracellular domain residues. E, *Eco*RI site; X, *Xba*I site; H, *Hae*II site; A, *Apa*I site: Arrows indicate direction of transcription.

METHODS. The CD43 cDNA clone HPB2.5 was isolated from an HPB-ALL cDNA library and subcloned into the *Not*I and *Hin*dIII sites of πH3M to generate πH3M-CD43. A 2.8-kb *Xba*I fragment containing the CD43 cDNA was excised from πH3M-CD43 and ligated into the *Xba*I site of pFneo. An 8.4-kb clone containing the CD43 cDNA in the proper orientation with respect to the SFFV-LTR (pCD43Fneo) was isolated for further manipulation. To generate pΔCD43Fneo, pCD43Fneo was digested with *Eco*RI and *Apa*I to yield: 2.1-kb *Eco*RI-*Apa*I, 1.2-kb *Apa*I, and 5.1-kb *Apa*I-*Eco*RI fragments. The 2.1-kb and 1.2-kb fragments were further digested with *Hae*II and the resulting 2.1-kb *Eco*RI-*Hae*II and 0.9-kb *Hae*II-*Apa*I fragments were purified and religated to the 5.1-kb *Apa*I-*Eco*RI fragment. The net result was the deletion of a 0.3-kb *Hae*II fragment encoding for 122 of the 123 intracellular amino acids. (The presence of multiple *Hae*II sites within the 5.1-kb *Apa*I-*Eco*RI fragment necessitated this approach.) Because this deletion also removed the stop codon of the original peptide-coding sequence, a new stop codon beginning 49 base pairs 3' to the ligation site was recruited, resulting in the addition of 16 new amino acids (LGRAGLAPLNVCGLNE, single-letter notation), none of them capable of being phosphorylated.

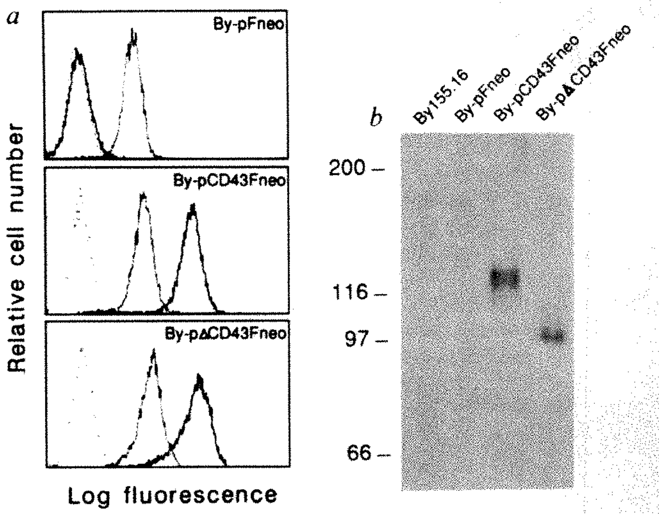


FIG. 2 Phenotype of transfected cells. *a*, Surface expression of the murine TCR (dotted line) and the extracellular domain of CD43 (solid line) on clones transfected with pFneo, pCD43Fneo and pΔCD43Fneo was assessed by indirect immunofluorescent staining using F23.1 (anti-TCR monoclonal antibody) or L10 (anti-CD43 monoclonal antibody) and FITC-conjugated goat anti-mouse immunoglobulin antibody. The anti-immunoglobulin antibody was used alone as a control (highly dotted line). The three TCR-CD3$^+$ clones shown were obtained by limiting dilution cloning. Not shown are the CD43$^-$, CD43$^+$, and ΔCD43$^+$ clones which were TCR-CD3$^-$. *b*, The relative molecular mass (M_r) of the proteins was determined by western blot analysis using L10 monoclonal antibody.

METHODS. The plasmids described in Fig. 1 were linearized with *Eco*RI, purified, and quantitated. By155.16 cells (2×10^7 ml^{-1}) were transfected by electroporation with one of the three plasmids (20 μg ml^{-1}) using standard protocols[25]. To generate clones, transfected cells were cultured at an initial density of 10^5 cells ml^{-1} of RPMI 1640 containing 20% heat inactivated FBS, 100 U ml^{-1} penicillin, 100 μg ml^{-1} streptomycin, and 50 μM 2-mercaptoethanol (20% FBS-complete medium). Forty-eight hours later, cells were transferred to 10% FBS-complete medium containing 3 mg ml^{-1} of G418. After 7 days of drug selection, cells were cloned by limiting dilution in 10% FBS-complete medium. Standard indirect immunofluorescent staining of clones to assess TCR and CD43 expression levels used F23.1 or L10 monoclonal antibody respectively. Western blot analysis of proteins used L10 monoclonal antibody.

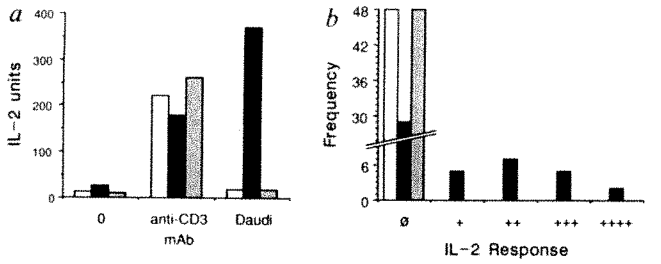


FIG. 3 Stimulation of transfected TCR–CD3$^+$ cells. *a*, The CD43$^-$ (□), CD43$^+$ (■), and ΔCD43$^+$ (▨) clones were stimulated with medium alone (○), the anti-CD3 monoclonal antibody 145-2C11, or Daudi cells. IL-2 production was measured as described below. Results from a single representative experiment repeated five times are shown. *b*, Individually derived CD43$^-$ (□, n=48), CD43$^+$ (■, n=48), and ΔCD43$^+$ (▨, n=48) cell lines were assessed for their IL-2 production in response to Daudi cell stimulation. The IL-2 values were measured and scored on an increasing [0] to [++++] scale as defined below. The number of cell lines generating a given IL-2 response is shown on the ordinate.

METHODS. For the generation of nonclonal cell lines, cells were transfecte as described in Fig. 2 and immediately transferred into 24-well tissue culture plates at a density of 10^5 cells per 1.5 ml of 20% FBS-complete medium Forty-eight hours later, 0.5 ml of 12 mg ml^{-1} G418 was added to each wel to achieve a final concentration of 3 mg ml^{-1}. After 7–10 days of drug selection, viable cell lines were chosen and cultured in G418-free 10% FBS-complete medium for an additional 7–10 days. Cells from each cell line were then analysed for surface expression of CD3 and CD43 as describe for the clones. Stimulation of clones and cell lines was performed in 24-wel tissue culture plates containing 1.0 ml total reaction volume. Responde cells were used at a density of 5×10^4 cells ml^{-1}. (anti-CD3 monoclona antibody was used at 1 μg ml^{-1}.) and irradiated (50 Gy) Daudi cells were used at a density of 5×10^5 cells ml^{-1}. After 24 h stimulation, supernatants from cultured cells were assayed for IL-2 content by their ability to promote the proliferation of an IL-2 dependent murine T cell line, CTLL 20. Proliferation was assessed by measuring the incorporation of [^{3}H]thymidine during the final 4 h of a 24-h incubation. Relative IL-2 units were determined for each supernatant by comparison with the proliferation of CTLL 20 supported by a standard rat concanavalin A supernatant, with half-maximal incorporation defined as 100 units. The cell lines were additionally assigned a response value based on the following scale: 0, <50 units; +, 50–80 units; ++ 80–110 units; +++, 110–150 units; ++++, >150 units.

whereas preincubation with liposomes containing purified HLA-DR had no effect (Fig. 4*a*). To demonstrate further the specificity of the CD43–Daudi cell interaction, wells coated with either CD43 or LFA-3 were preincubated with either anti-CD43 (B1B6 or G10-2, gifts of B. Axelsson and J. Ledbetter, respectively), anti-CD2 (TS2/18), or anti-LFA-3 (TS2/9) monoclonal antibodies. The plates were washed to remove unbound antibody, and Daudi cell adhesion assessed. B1B6 and G10-2 inhibited Daudi cell adhesion by 63% and 57%, respectively (Fig. 4*b*). In contrast, neither B1B6 nor G10-2 could inhibit the binding of a CD2$^+$ hybridoma to plate-bound LFA-3, binding that could be inhibited by an anti-LFA-3 monoclonal antibody (TS2/9) (data not shown). Daudi binding to LFA-3 was not modified by the addition of anti-CD43 monoclonal antibodies (data not shown). These data strongly suggest that the CD43 molecule can bind to a ligand on the surface of Daudi cells. The avidity and specificity of this interaction are great enough to mediate the selective adherence of Daudi cells to immunoaffinity-purified CD43. In addition, this interaction can be inhibited by the anti-CD43 monoclonal antibodies B1B6 and G10-2, but not by control antibodies.

The data presented here demonstrate directly that in the presence of TCR-CD3 expression, CD43 expression by T cells enhances their response to antigen. The existence on antigen-presenting (Daudi) cells of a specific ligand for CD43 is strongly implied by the data. Following the engagement of a prospective

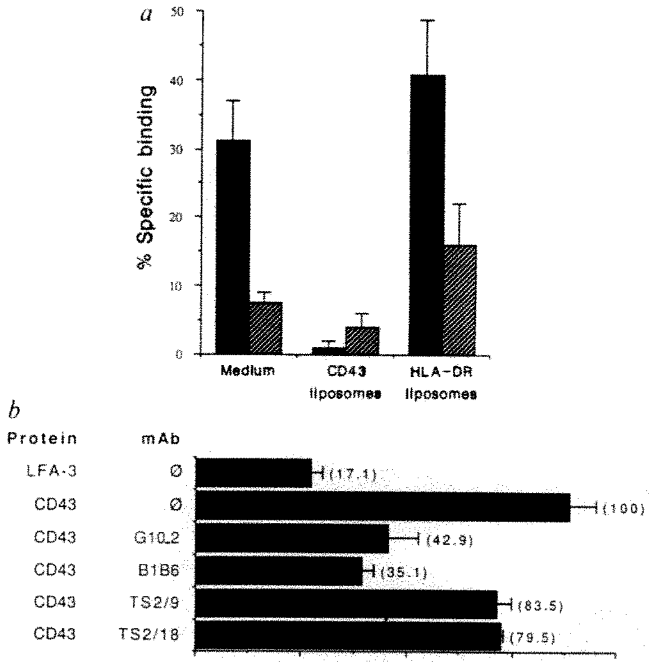


FIG. 4. Binding of human and murine B cells to human CD43. *a*, The specific binding of Daudi (■), a human B cell line, and A20 (▨), a murine B cell line, to CD43-adsorbed surfaces was assessed. Both cell types were preincubated with 10% FBS-complete medium containing either no liposomes, CD43-liposomes, or HLA-DR-liposomes. *b*, The binding of Daudi cells to CD43-adsorbed surfaces was inhibited by the anti-CD43 monoclonal antibodies B1B6 and G10-2. Data are expressed in c.p.m. The specific binding of Daudi cells to CD43 was assigned the value of 100%, and the binding of Daudi cells in the presence of monoclonal antibodies is expressed as a percentage of this binding (values in parentheses). The experiment shown is representative of five such experiments. Statistical analysis of all five assays revealed that the inhibition of Daudi cell binding to CD43 by anti-CD43 mAbs (B1B6 and G10-2) was significant ($P < 0.001$) and that the control anti-CD2 (TS2/18) and anti-LFA3 (TS2/9) monoclonal antibodies had no effect on binding ($P > 0.5$ and $P < 0.2$ respectively).

METHODS. CD43 was immunoaffinity purified from ^{125}I-labelled and unlabelled JY cell lysates using an L10 mAb-conjugated sepharose 4B column. PAGE of the isolated proteins followed by Coomassie blue staining and autoradiography, silver staining, or western blotting all revealed a single band of M_r 115,000, confirming the identity and purity of the sample. To a 96-well plate were added 50 μl of a 2.5 ng ml^{-1} solution of purified CD43 in 0.3% octyl-β-glucoside, 0.1 M sodium bicarbonate buffer (pH 9.2). Equivalent amounts of immunopurified LFA-3[26] were added to control wells under identical conditions. Plates were incubated for 3 h at 37 °C, after which the remaining protein binding sites were blocked with 1% BSA for 2 h at 37 °C. Wells were extensively washed to remove unadsorbed proteins and [^{3}H]-thymidine-labelled cells were then added to the plates at a density of 2×10^5 cells per well. The plates were centrifuged at 50 g for 1 min and placed in a 37 °C, 5% CO_2-in-air environment for 30 min. Unbound cells were then removed by aspirating the fluid contents of each well through an 18-gauge needle and carefully rinsing each well four times with 200 μl of prewarmed (37 °C) 10% FBS-complete medium. After washing, the radioactivity contained in each well was measured as c.p.m. Also determined were the total c.p.m. of cells initially added to the wells. The mean percentage of cells bound to BSA (alone)-adsorbed wells was 9.8 ± 4.7 (n=5 assays). Percentage specific binding was calculated as the: ((c.p.m. of cells retained by CD43-adsorbed wells) − (c.p.m. of cells retained by BSA-adsorbed wells)) ÷ ((total c.p.m. of cells added to the wells) − (c.p.m. of cells retained by BSA-adsorbed wells)). For the liposome inhibition assays, cells were preincubated for 1 h at 37 °C with 10% FBS-complete medium containing either no liposomes, CD43-liposomes, or HLA-DR-liposomes (1 μg protein per 20 nmol lipid) before plating. For the monoclonal antibody inhibition assays, protein-coated wells were incubated with a 1 μg ml^{-1} monoclonal antibody solution for 4–6 h at 37 °C; excess monoclonal antibody was removed before the addition of cells. Before addition to protein-coated wells, Daudi cells were incubated with anti-CD18 monoclonal antibody (TS1/18) to inhibit homotypic adhesions.

ligand through its extracellular domain, CD43 probably transduces a costimulatory signal through its intracellular domain. This signal, in synergy with a subthreshold signal provided by the TCR-CD3, leads to T-cell activation. Although a ligand has not yet been isolated, the data presented here should lead to a greater understanding of the role of CD43 in the pathogenesis of the Wiskott-Aldrich syndrome and in the activation of T cells. ☐

Received 20 December 1990; accepted 4 March 1991.

1. Gahmberg, C. G. & Andersson, L. C. *J. biol. Chem.* **252,** 5888–5894 (1977).
2. Gahmberg, C. G., Hayry, P. & Andersson, L. C. *J. Cell Biol.* **68,** 642–653 (1976).
3. Brown, W. R. A., Barclay, A. N., Sunderland, C. A. & Williams, A. F. *Nature* **289,** 456–460 (1981).
4. Remold-O'Donnell, E., Davis, A. E., Kenney, D., Bhaskar, K. R. & Rosen, F. S. *J. biol. Chem.* **261,** 7526–7530 (1986).
5. Carlsson, S. R. & Fukuda, M. *J. biol. Chem.* **261,** 12779–12786 (1986).
6. Axelsson, B., Hammerstrom, S., Finne, J. & Perlmann, P. *Eur. J. Immun.* **15,** 427–433 (1985).
7. Borche, L., Lozano, F., Vilella, R. & Vives, J. *Eur. J. Immun.* **17,** 1523–1526 (1987).
8. Remold-O'Donnell, E., Zimmerman, C., Kenney, D. & Rosen, F. S. *Blood* **70,** 104–109 (1987).
9. Parkman, R., Remold-O'Donnell, E., Kenney, D. M., Perrine, S. & Rosen, F. S. *Lancet* **ii,** 1387–1389 (1981).
10. Axelsson, B., Youseffi-Etemad, R., Hammerstrom, S. & Perlmann, P. *J. Immun.* **141,** 2912–2917 (1988).
11. Vargas-Cortes, M., Axelsson, B., Larsson, A., Berzins, T. & Perlmann, P. *Scand. J. Immun.* **27,** 661–671 (1988).
12. Beyers, A. D., Barclay, A. N., Law, D. A., He, Q. & Williams, A. F. *Immun. Rev.* **111,** 59–77 (1989).
13. Nong, Y.-H., Remold-O'Donnell, E., LeBien, T. W. & Remold, H. G. *J. exp. Med.* **170,** 259–267 (1989).
14. Wiken, M., Bjork, P., Axelsson, B. & Perlmann, P. *Scand. J. Immun.* **29,** 363–370 (1989).
15. Mentzer, S. J. et al. *J. exp. Med.* **165,** 1383–1392 (1987).
16. Pallant, A. *et al. Proc. natn. Acad. Sci. U.S.A.* **86,** 1328–1332 (1989).
17. Shelley, C. S. *et al. Proc. natn. Acad. Sci. U.S.A.* **86,** 2819–2823 (1989).
18. Sleckman, B. P. *et al. Nature* **328,** 351–353 (1987).
19. Chatila, T. A. & Geha, R. S. *J. Immun.* **140,** 4308–4314 (1988).
20. Axelsson, B. & Perlmann, P. *Scand. J. Immun.* **30,** 539–547 (1989).
21. Piller, V., Piller, F. & Fukuda, M. *J. biol. Chem.* **264,** 18824–18831 (1989).
22. Bierer, B. E., Sleckman, B. P., Ratnofsky, S. E. & Burakoff, S. J. *A. Rev. Immun.* **7,** 579–599 (1989).
23. Springer, T. A. *Nature* **346,** 425–434 (1990).
24. Ohashi, P. S. *et al. Nature* **316,** 606–609 (1985).
25. Potter, H. in *Current Protocols in Molecular Biology* (eds Ausubel, F. M. *et al.*) 9.3.1–9.3.2 (Wiley, New York, 1987).
26. Takai, Y., Reed, M. L., Burakoff, S. J. & Herrmann, S. H. *Proc. natn. Acad. Sci. U.S.A.* **84,** 6864–6868 (1987).

ACKNOWLEDGEMENTS. This work was supported by the NCI (S.J.B.), IAID (F.S.R.) and GMI (E.R.-O.). J.K.P. is the recipient of a Physician-Scientist Award and a Medical-Scientist Award. B.E.B. is the recipient of a Clinician-Scientist Award from the American Heart Association and a McDonnell Scholar Award from the J. S. McDonnell Foundation. We thank W. C. Hahn for support and helpful discussions.

HIV enhancer activity perpetuated by NF-κB induction on infection of monocytes

F. Bachelerie, J. Alcami, F. Arenzana-Seisdedos & J.-L. Virelizier*

Unité d'Immunologie Virale, Institut Pasteur, 28 rue due Dr Roux,
75724 Paris Cedex 15, France

PERMISSIVENESS to replication of human immunodeficiency virus (HIV) differs in T lymphocytes and macrophages. In T cells, HIV transcription is poorly detected *in vivo*[1]. Cloned, normal T lymphocytes show very little, if any, basal activity of the HIV enhancer and low nuclear expression of NF-κB[2], a potent transcriptional activator of the HIV enhancer[3–5]. In contrast, fixed tissue macrophages express detectable HIV proteins, indicating permanent virus transcription[6]. One explanation for the perpetuation of virus infection in macrophages could be sustained nuclear NF-κB expression. However, the U937 monocytic cell line, which is fully permissive to HIV replication, is known to express only low levels of nuclear NF-κB[7]. We show here that chronic HIV infection results in both induction of a nuclear factor with antigenic properties indistinguishable from those of NF-κB and permanently increased HIV enhancer activity. This phenomenon, which is independent of tumour necrosis factor, is associated with HIV replication, and is thus likely to explain at least in part the perpetuation of HIV infection in monocytes.

To test the hypothesis that HIV replication induces a functional transcription factor(s) which can perpetuate HIV genome transcription, we analysed the activity of HIV enhancer-driven vectors in chronically infected U937 cells. Because the basal expression of nuclear NF-κB varies according to experimental conditions[8], we maintained cell cultures in the exponential growth phase and avoided all endotoxin or mycoplasma contamination. Figure 1*a* shows reverse transcriptase (RT) production by U937 cells which began at day 11 in one representative experiment (of 10) shown. Neutralization of input virus by soluble CD4 (sCD4) produced a 20-day delay in virus replication. Figure 1*b* shows that nuclear protein(s) were detected in infected cells which bound to an oligonucleotide with the sequence of the HIV enhancer, but not to an oligonucleotide with a mutation which abolishes NF-κB binding[3]. This activity was barely detectable in uninfected cells, although a longer exposure of autoradiographs revealed a low-intensity pattern

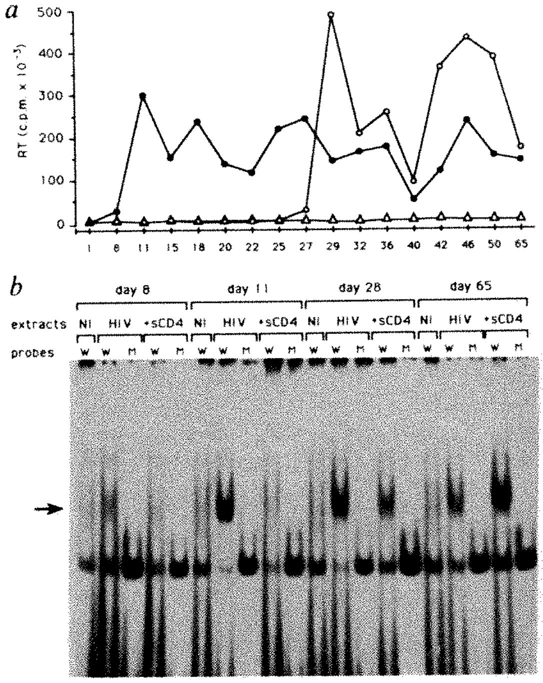
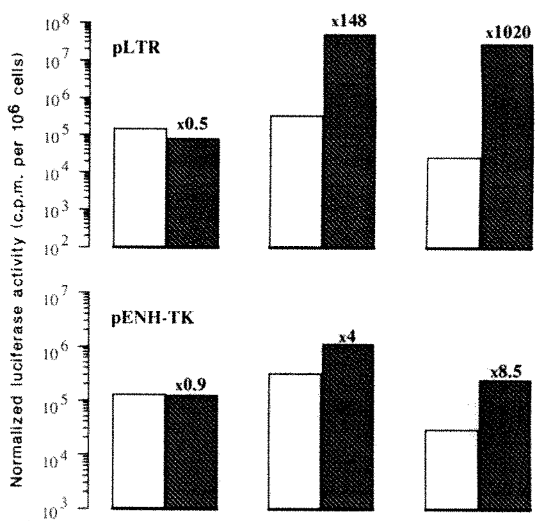


FIG. 1 Induction of nuclear HIV enhancer-binding proteins during HIV infection of monocytic U937 cells. *a*, Kinetics of reverse transcriptase (RT) activity in supernatants of U937 cells infected with HIV in the absence (●) or presence of soluble recombinant CD4 (American Biotechnology) (sCD4) (○) or mock-infected (△). The U937 cell line showed the following phenotype: Fcγ-receptor-, CD11a-, CD11b-, CD4-positive, CD3-, CD14-, CD25-negative and non-adherent. Cells were incubated for 1h with a preparation of HIV LAV-1 Bru strain grown in U937 which was clarified, filtered (0.2 μm pore size) and used at a concentration of 6×10^5 c.p.m. of RT per 1×10^6 cells per ml. Mock-infected cells received RPMI-1640 medium supplemented with 5% FCS. No polybrene was added. Neutralization with sCD4 (50 μg ml^{-1}) of 1 ml virus stock (6×10^6 c.p.m. of RT) was performed for 1h at 4°C before addition to 9 ml cell suspension for 1 h at 37 °C. All cells were then washed three times with PBS and maintained at 37 °C, in a 5% CO_2 atmosphere at a concentration of 2.5×10^5 per ml of culture medium (RPMI supplemented with 10% FCS). RT activity (c.p.m. per 50 μl) was measured by standard microassay[17]. *b*, Bandshift assays with nuclear extracts (5 μg) from infected cells in the absence (HIV) or presence of sCD4 (+sCD4) or control cells (NI) at days 8, 11, 28 and 65 after infection were prepared as previously described[2,18]. The binding probes were end-labelled double-strand synthetic oligonucleotides corresponding to the wild-type NF-κB sequence of the HIV enhancer (W) (gift from C. Auffray and A. Delaroche): 5'-ACAAGGGACTTTCCGCTGGGGACTTTCCAGGGA-3', or the mutated HIV enhancer sequence[3] (M): 5'-ACAACTCACTTTCCGCTGCTCACTTTCCAGGGA-3' where mutated sites are underlined. Arrow denotes specific binding. Autoradiography was for 5 h.

---

* To whom correspondence should be addressed.

Days after infection	3		22		28	
HIV infection	-	+	-	+	-	+



p24 (ng ml^{-1})	<.05	<.05	<.05	3500	<.05	4600
RT (c.p.m.x 10^3)	5	4	2	260	2	360
in situ (% + cells)	0	0	0	82	0	86

FIG. 2 Activity of the HIV LTR- or HIV enhancer-TK-luciferase expression vectors transfected into infected (▨) or control (☐) U937 cells at days 3, 22 and 28 after infection. The HIV LTR-luciferase vector was the *XhoI* (−640)-*HindIII* (+78) fragment containing the U3 + R regions from the LAV-1 Bru strain cloned into the pC-luc plasmid[19] generating pLTR, as described elsewhere[19]. The Enh-TK-luciferase vector containing the HIV-enhancer sequence was constructed by cloning the 270-basepair (bp) *BglII* (Klenow)-*SalI* fragment of plasmid pHI-03TK-CAT[18] into the *HindII*- (Klenow)*SalI* sites of the pC-luc polylinker[19]. This vector contains three copies of the synthetic HIV-enhancer oligonucleotide described in Fig. 1 as (W), cloned upstream of the weak, truncated Herpes simplex thymidine kinase (TK) promoter (−105-+51). Transfection was performed according to a modified DEAE-dextran technique[20] using 5 μg plasmid DNA per 1.5×10^7 cells. Luciferase activity was measured[19] in cell lysates made 60 h after transfection and is expressed as c.p.m. per 1×10^6 living cells, after normalization in function of the relative activity of a pTK-luciferase vector transfected into control (☐) or infected (▨) cells. Comparable amounts of transfected plasmid were found by DNA Hirt extraction[21] 2 h after transfection in control and infected cells and input DNA plasmid correlated with luciferase expression of the TK-luciferase plasmid. The pTK-luciferase vector was constructed by ligation of the *PvuI-BglII* fragment containing the TK promoter from pBL-CAT2[22] vector with the *PvuI-BamHI* fragment of pC-luc. Numbers at the top of bars represent fold amplification of luciferase activity in infected cells compared to that expressed by the same vector in control cells in the same experiment. RT assay was performed and expressed as described in Fig. 1. Levels of p24 in culture supernatants are indicated as nanograms per millimetre, based on p24 standards (NEN Research product). For *in situ* detection[23,24], an HIV (LAV-1 Bru) *gag* insert (*SacI* (224)-*HindIII* (2078)) was cloned into a pSP72 vector which provides SP6/T7 promoters (Promega-Biotech). RNA probes were generated with either Sp6 (sense) or T7 (antisense) RNA polymerase and hybridized *in situ* with control or infected cells. Results are expressed as % of positive cells with the T7 (antisense) *gag* probe. When positive cells were observed, the percentage was evaluated on at least 500 cells. Negative results were recorded after screening of at least 10^4 cells.

similar to that in Fig. 4. This NF-κB-like factor was not seen in the nuclei of U937 cells transiently protected from infection by sCD4, but was observed (day 28) following escape from protective neutralization of the input virus. Chronically infected cells remained positive for both RT production and nuclear NF-κB expression for the ten months duration of this analysis. These results were observed after infection with HIV stocks grown in either CEM or U937 cells and with either crude or sucrose gradient-purified virus.

We recently observed in human T cell clones, that translocation of NF-κB into the nucleus is not necessarily sufficient to induce transcription dependent on HIV enhancer[2]. We therefore transiently transfected chronically infected cells with a series of luciferase expression vectors under the control of various regions of the LAV-1 Bru long terminal repeat (LTR). HIV-infected cells showed enhanced activity of an expression vector under the control of three copies of an oligonucleotide with the sequence of the two 10-base pair direct repeats of the HIV enhancer (pENH-TK) (Fig. 2). This was only seen after detectable virus replication (day 22) at a stage when more than 80% of cells were actively producing HIV *gag* transcripts as shown in the two representative *in situ* hybridization experiments performed 22 and 28 days after the onset of infection. Strong NF-κB like nuclear binding activity was detected in infected, but not in control, cells on day 22 (Fig. 4*a*). Transactivation in cells replicating HIV was similar to that in control cells stimulated with phorbol myristate acetate (PMA) (4–15-fold and 1.3–9.6-fold amplifications, respectively). The activity of a vector under the control of the whole (U3 + R) HIV LTR (pLTR) in infected cells was much greater than that of the enhancer-driven vector, probably reflecting HIV *tat* protein function, because deletion of the TAR sequence led to a response comparable in intensity to that of the enhancer-TK-luciferase vector (Fig. 3). Similarly, an LTR fragment (−158–69) including the HIV enhancer motif and flanking sequences cloned upstream of the TK promoter (pF2-TK) transfected into infected cells showed an increased activity, comparable with that of the enhancer-driven vector. Such increased activity was not seen in infected cells transfected with a vector under the control of an oligonucleotide mutated at bases critical for binding of NF-κB (Fig. 3). Therefore, the protein factor in chronically infected cells recognizes the NF-κB consensus motif and is functional. Interestingly, a modest but

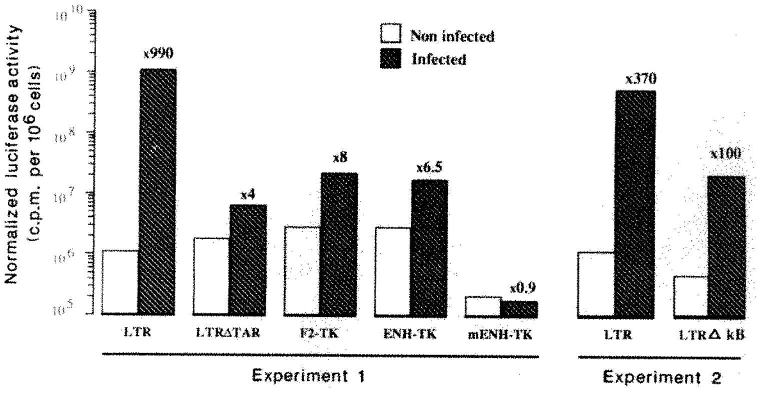
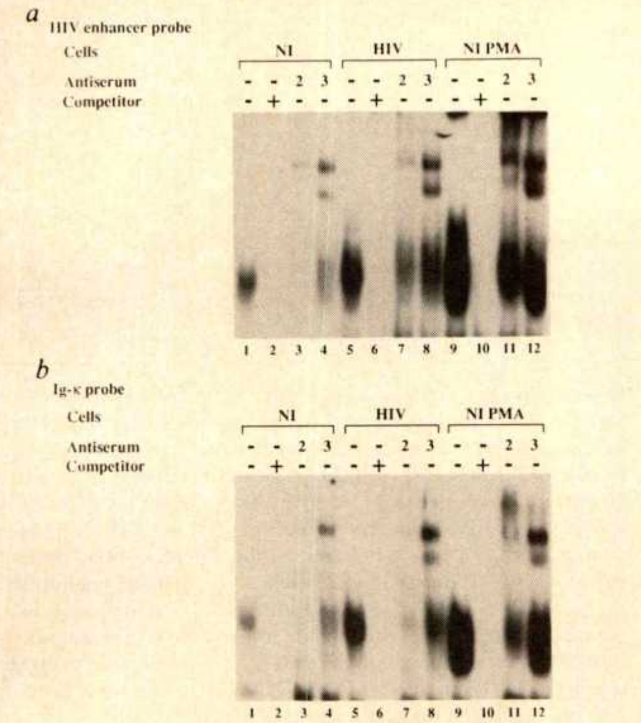


FIG. 3 Activity of LAV-1 Bru LTR luciferase expression vectors transfected into infected (▨) or control (☐) U937 cells. The ENH-TK luciferase construct is described in Fig. 2. The HIV LTR vector contains the *BglII* (−485)-*HindIII* (+78) fragment. The LTRΔTAR vector contains the *BglII* (−485)-*SacI* (+38) LTR fragment deleted of the TAR region and cloned into the pC-luc plasmid. The F2-TK-luciferase vector was the (−158-69) *HaeIII* fragment of the HIV LTR[18] containing the HIV-enhancer sequence cloned upstream of the TK promoter. The mENH-TK luciferase construct contains four copies of a mutated synthetic HIV-enhancer oligonucleotide (described in Fig. 1 as M) cloned upstream the TK promoter. The LTRΔκB was obtained by insertion into the pC-luciferase plasmid[19] of the κB consensus motifs-deleted *Xho-HindIII* fragment of the Lav-1 Bru LTR from the original CAT construct of A. Rabson[25]. Transfection, luciferase activity detection and normalization were performed as described in Fig. 2. Numbers at the top of bars represent fold amplification of luciferase activity in infected cells compared to that expressed by the same vector in control cells in the same experiment.



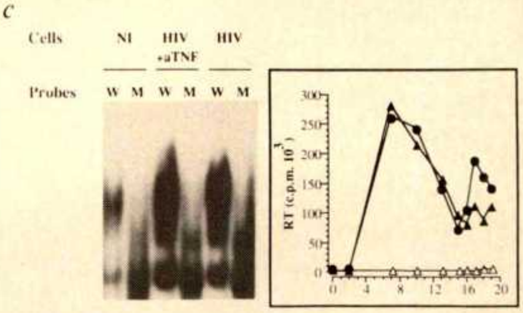


FIG. 4 Binding properties of specific nuclear factor(s) detected in uninfected and infected cell extracts. Assays conditions as described in Fig. 1. *a*, Bandshift assay was performed using the Klenow-labelled, double-stranded oligonucleotide corresponding to the HIV-enhancer sequence. Nuclear extracts (2.5 μg) were made from uninfected cells (lanes 1–4), from 22 day-infected cells (lanes 5–8) (LTR activity shown in Fig. 2) and from cells incubated for 16 h with PMA (lanes 9–12). Lanes 1, 5 and 9 extracts were incubated without competitor. Lanes 2, 6 and 10 extracts were incubated with an 80-fold excess (20 ng) of the unlabelled HIV-enhancer probe. Reactivity of nuclear extracts with antisera obtained from rabbit immunized with purified KBF-1 fusion protein included glutathione transferase linked to amino acids 19–969 of KBF1[9] (antiserum 2, previously described[9]) (lanes 3, 7 and 11) and to amino acids 19–502 (antiserum 3, previously described[9]) (lanes 4, 8 and 12) (kindly provided by A. Israël). As previously described[9], 0.6 μl of whole antiserum was included in a 20 μl bandshift assay, together with nuclear extracts. Autoradiography was for 16 h. *b*, Bandshift assay using an end-labelled, double-stranded oligonucleotide corresponding to the immunoglobulin κ chain NF-κB motif (previously described[18]). Nuclear extracts from non-infected cells (lanes 1–4), from 40 day-infected cells (lanes 5–8) (LTR activity shown in Fig. 3), from cells incubated for 16 h with PMA (lanes 9–12). Lanes 1, 5 and 9 were incubated without competitor. Lanes 2, 6 and 10 were incubated with an 80-fold excess (20 ng) of the unlabelled HIV-enhancer probe. Lanes 3, 7 and 11, assays contained antiserum 2 and lanes 4, 8 and 12 received antiserum 3. *c*, (left panel) Bandshift assay of a Klenow-labelled double-stranded oligonucleotide corresponding to the HIV-enhancer sequence (W) or a mutated HIV enhancer sequence (M) using nuclear extracts made on day 19 after infection from infected cells cultured with 1 μg per ml of neutralizing anti-TNF monoclonal antibody (HIV +aTNF) or with a control antiserum (HIV) or from uninfected cells (NI). Right panel, kinetics of reverse transcriptase (RT) activity in supernatants of U937 cells infected with HIV (LAV-1 Bru strain) cultivated in presence of antibody to TNF (▲), in presence of a control antiserum (●) or mock-infected (△).

consistent difference in activity was seen between the enhancer- and the mutated oligonucleotide-driven vectors in control, uninfected cells. Therefore, the binding activity in control cells after sufficient autoradiographic exposure (see Fig. 4*a* and *b*) is also functional.

The bandshift assays in Fig. 4 indicate that the HIV enhancer binding complex was specifically competed by an oligonucleotide with the NF-κB consensus motif in the context of the HIV enhancer itself and detected with both an HIV enhancer (Fig. 4*a*) and an immunoglobulin κ chain enhancer probe (Fig. 4*b*). Addition of specific antibody to purified recombinant p50[9] caused a profound decrease and partial upshift (depending on the antiserum used) of the binding observed in nuclear extracts from infected, uninfected and PMA-stimulated cells. We have confirmed (A. Israël, personal communication) that antiserum number 2 does not immunoprecipitate the c-rel protein translated *in vitro* from c-*rel* DNA (gift from N. Rice). Preliminary experiments have also shown that the binding observed in infected cell nuclei is not influenced by an antiserum to the C-terminal part of the c-rel protein (gift from I. Verma and L. Kerr) which does not show homology with the p50 protein of the NF-κB complex. Thus, the protein complex showed migration and antigenic properties indistinguishable from those of NF-κB[9,10].

The mechanisms through which HIV infection leads to NF-κB induction in U937 cells is not clear. Contamination of our viral preparations by non-viral products such as lymphokines or endotoxins was controlled by sCD4 neutralization assays. Direct induction by binding of viral particles to membrane CD4, a transducing molecule[11–13], is unlikely because excess sucrose gradient-purified, viable LAV-1 Bru particles did not induce NF-κB 5–48 h (not shown) or 8 days (see Fig. 1) after infection. Autocrine secretion of tumour necrosis factor (TNF) was also ruled out (Fig. 4*c*). TNF-neutralizing antibody in the culture medium did not influence RT production or NF-κB induction. Moreover, TNF and interleukin 1 activities were not detected (not shown). The mechanism(s) of NF-κB induction during chronic infection of U937 cells is unknown, but seems to be associated with HIV replication. For example, proteolysis may regulate NF-κB binding activity[14] during HIV infection because others (A. Israël, personal communication) have observed that the HIV protease influences the maturation and potentially the translocation, of NF-κB.

We have shown that HIV infection itself upregulates a low constitutive nuclear expression of NF-κB in U937 cells. Therefore, HIV infection may be self-perpetuating in monocytic cells. HIV latency is presumably the result of low expression of nuclear factors critically involved in LTR-dependent transcription[15]. Following *tat* production after HIV LTR-activation, an amplification loop allows full HIV transcription and replication. Recent evidence indicates that the HIV enhancer domain is critical for HIV *tat* functioning[16]. Figure 3 (experiment 2) provides further evidence that tat function is decreased when NF-κB consensus motifs are deleted in the LTR. The activity of the ΔκB vector compared to that of the wild-type LTR was decreased by a factor of 20 (19×10^6 versus 390×10^6 c.p.m. per 10^6 cells of normalized luciferase activity, respectively) in infected cells. This is consistent with the finding[26] that replication in U937 of a ΔκB-deleted provirus is diminished but not abolished. These data indicate that the κB sequences of the LTR participate clearly, although not solely, in the optimal transactivating effects of tat. Therefore HIV replication itself, through induction of an increased HIV enhancer activity, may initiate and perpetuate the tat-mediated amplification of HIV genome transcription, thus modifying the monocyte cell environment to its own advantage. ☐

Received 5 December 1990, accepted 6 March 1991.

1. Poli, G. *et al. Science* **244,** 575-577 (1989).
2. Hazan, U. *et al. Proc. natn. Acad. Sci. U.S.A.* **87,** 7861-7865 (1990).
3. Nabel, G. & Baltimore, D. *Nature* **326,** 711-713 (1987).
4. Siekewitz, M. *et al. Science* **238,** 1575-1578 (1987).
5. Kaufman, J. D. *et al. Molec. cell. Biol.* **7,** 3759-3766 (1987).
6. Gendelman, H. E. *et al. AIDS* **3,** 475-495 (1989).
7. Griffin, G. E. *et al. Nature* **339,** 70-73 (1989).
8. Griffin, G. E. *et al. Nature* **343,** 219 (1990).
9. Kieran, M. *et al. Cell* **62,** 1007-1018 (1990).
10. Ghosh, R., *et al. Cell* **62,** 1019-1029 (1990).
11. Merrill, J. E., Koyanagi, I & Chen, I. S. *J. Virol* **63,** 4404-4408 (1989).
12. Poli, G. *et al. Proc. natn. Acad. Sci. U.S.A.* **87,** 782-785 (1990).
13. Wahl, L. *et al. Proc. natn. Acad. Sci. U.S.A.* **86,** 621-625 (1989).
14. Gilmore, T. D. *Cell* **62,** 841-843 (1990).
15. Pomerantz, R. J., Trono, D., Feinberg, M. B. & Baltimore, D. *Cell* **61,** 1271-1276 (1990).
16. Berkhout, B., Gatignol, A., Rabson, A. B. & Jeang, K-T. *Cell* **62,** 757-767 (1990).
17. Schwartz, O., Henin, Y., Marechal, V. & Montagnier, L. *AIDS Res. hum. Retrovir.* **4,** 441-448 (1988)
18. Israël, N. *et al. J. Immun.* **143,** 3956-3960 (1989).
19. Schwartz, O., Virelizier, J-L., Montagnier, L. & Hazan, U. *Gene* **88,** 197-205 (1990).
20. Wang, Y., Larsen, A. S. & Peterlin, B. M. *J. Exp. Med.* **166,** 625-637 (1987).
21. Hirt, R. *J. Molec. Biol.* **26,** 365-369 (1967).
22. Luckow, B. & Schütz, G. *Nucleic Acids Res.* **15,** 5490 (1987).
23. Bachelerie, F. *et al. J. Virol.* **69,** 3059-3062 (1990).
24. Haase, A., Brahic, M., Stowing, L. & Blum, H. *Methods Virol.* **7,** 189-226 (1984).
25. Du, E. J., Maury, W. J., Folks, T. M., Fauci, A. S. & Rabson, A. B. *Proc. natn. Acad. Sci. U.S.A.* **86** 5974-5978 (1990).
26. Harrich, D., Garcia, J., Mitsuyasu, R. & Gaynor, R. *EMBO J.* **9,** 4417-23 (1990).

ACKNOWLEDGEMENTS. We thank A. and N. Israël, F. Logeat and S. Michelson for helpful discussions A. Israël for antibody number 2 and 3 to p50[9] and C. Auffray for oligonucleotide synthesis. We thank D. Thomas and F. Aillet for technical assistance. The Agence Nationale de Recherche sur le Sida (A.N.R.S) supported this work. We are grateful for a fellowship from A.N.R.S to F.B and from Caja de Ahorros de Madrid (Spain) to J.A.

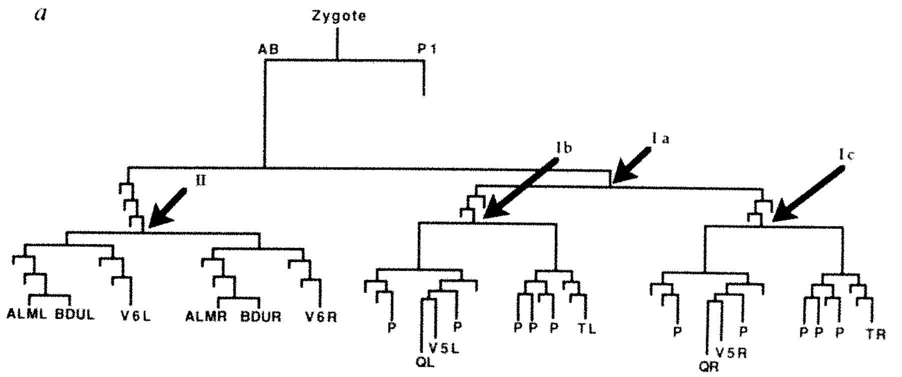
Regulation of cellular responsiveness to inductive signals in the developing *C. elegans* nervous system

David A. Waring & Cynthia Kenyon

Department of Biochemistry and Biophysics, University of California, San Francisco, California 94143-0554, USA

IN *Caenorhabditis elegans*, cell–cell communication is required to form a simple pattern of sensory ray neurons and cuticular structures (alae). The *C. elegans pal-1* gene initiates one developmental pathway (ray lineages) simply by blocking a cell–cell interaction that induces an alternative pathway[1]. Here we show by mosaic analysis that *pal-1*$^+$ acts by preventing specific cells from responding to inductive signals. The results indicate that although cell signals play a critical role in generating this pattern, they do not provide spatial information. Instead, signals are sent to many, if not all, of the precursor cells, and the ability to respond is spatially restricted. This patterning strategy thus differs from many well known models for pattern formation in which localized inductive signals influence a subset of cells within a field. We find that *pal-1* encodes a homeodomain protein and so is likely to regulate transcription. The *pal-1*$^+$ protein could block the response to cell signals either by repressing genes involved in signal transduction or by acting directly on downstream genes in a way that neutralizes the effects of the intercellular signals. Genetic experiments indicate that one candidate for such a downstream gene is the *Antennapedia*-like homeotic selector gene *mab-5*.

In a newly hatched *C. elegans* male, seven cells (V1-V6 and T) lie in a row along each side of the animal[2]. Signals from neighbouring V cells induce V cells located in anterior body regions to generate epidermal lineages ('alae lineages'), including cells that produce alae[3]. Posterior V and T cells generate



b

Class	No.	ALML	BDUL	QL.pa	V5L.pa	V6L	ALMR	BDUR	QR.pa	V5R.pa	V6R	Pn.a
Ia	3	+	+	Ncl	Ncl	non-Pal	+	+	Ncl	Ncl	non-Pal	--
b	2	+	+	Ncl	Ncl	non-Pal	+	+	+	+	non-Pal	1/2 Ncl
c	1	+	+	+	+	non-Pal	+	+	Ncl	Ncl	non-Pal	1/2 Ncl
II	12	Ncl	Ncl	+	+	Pal-1	Ncl	Ncl	+	+	Pal-1	--

FIG. 1 Mosaic analysis of *pal-1*. *a*, Lineage diagram showing the relationships between V5, V6, T and the cells in which the Ncl-1 phenotype was used to score for the presence of the free duplication (see methods below)[9]. The arrows identify the point of loss of the duplication in various classes of mosaics. In the cases where the point of duplication loss is ambiguous, the arrow points to the last cell in which the loss could have occurred. The diagram represents only lineal relationships between cells, and not the relative timing of cell divisions. *b*, Summary of the Ncl and Pal-1 phenotypes observed in different classes of mosaic animals.

METHODS. Mosaic animals arose spontaneously in the strain *sDp3*; *pal-1 (e2091) dpy-17(e164) ncl-1(e1865) unc-36(e251)*; *him-5(e1490)*. The free duplication *sDp3*, which carries wild-type copies of these linked genes, is lost in roughly 1/400 cell divisions[4,10]. The mutation *ncl-1(e1865)* causes cells to have unusually large nucleoli. This gene has been shown to act cell-autonomously and thus allows one to determine cell genotypes (E. Hedgecock, personal communication). The Ncl phenotype is especially visible in neuronal cells. Because we were not able to score the Ncl-1 phenotype in all cells directly, the genotype of some cells was deduced by scoring lineally related cells in order to determine in which cell the duplication was lost during development. Classes Ia, Ib, and Ic: these animals were identified on the basis of the Ncl-1 phenotype of Q-derived neurons (Q.paa and Q.pap), the P(1-10).a-derived cells and the V5.pa descendants (the postdeirid). Animals were generally examined in early L3 for the Ncl-1 phenotype and then scored as young adults for the Pal-1 phenotype. Animals were classified as class Ia mosaics if the Q-derived cells and postdeirid cells on both sides of the animal were Ncl and the ALM and BDU cells on both sides were non-Ncl. Animals were placed in class Ib or Ic if the ALM and BDU cells on both sides were non-Ncl, Q-derived cells and the postdeirid cells on one side were Ncl and on the other side were non-Ncl, and Pn.a-derived cells from one of each bilateral pair were Ncl (either P1 or P2, P3 or P4, and so on). Class II: two class II animals were identified by scoring the Ncl phenotype in L3 animals. The remaining class II animals were found as adult males exhibiting a Pal-1 phenotype (both sides) but not a Dpy Unc phenotype (indicating that they carried the duplication in some cells), and then scored for the Ncl phenotype. Eleven such animals were identified. Ten of these had Ncl ALM and BDU cells (both sides), indicating a loss between ABa and ABappp. One animal did not exhibit a Ncl phenotype (we scored neurons and coelomocytes), and so may have been a recombinant or a double loss. The absence of other Ncl patterns among these phenotypically Pal-1 animals is significant, because it suggests that losses in other lineages do not produce a Pal-1 phenotype. But it should be noted that we have not tested all lineages explicitly. One animal scored as Pal had a partial Pal-1 phenotype: one side had no V6 rays while the other had 3 V-derived rays. Such partial phenotypes are found in ~15% of the animals in a *pal-1* homozygous strain[1].

different lineages ('ray lineages') that consist of ray neuroblasts as well as epidermal cells[2]. The gene *pal-1* (for 'posterior alae') is required for V6 to generate its characteristic ray lineage instead of alae lineages. The V6 ray-to-alae transformation in *pal-1* mutants is the consequence of inappropriate cell communication: ablation of V6's neighbour, T, allows V6 to generate its normal ray lineage in spite of the *pal-1* mutation. Thus the function of wild-type *pal-1* is to block or override signals from T that would otherwise induce V6 to produce alae lineages (see Fig. 4*a*). This implies that pattern formation among these cells involves the localized inhibition of cell interactions[1].

Wild-type *pal-1* activity could prevent V6 from communicating effectively with T either by preventing T from sending signals or by preventing V6 from receiving signals. To distinguish between these possibilities, we generated genetic mosaic animals that were genotypically *pal-1*$^+$ in some cells and *pal-1*$^-$ in others. These animals were isolated from a strain that carried a *pal-1*$^-$ mutation on each chromosome and a wild-type *pal-1*$^+$ gene copy on a free duplication. During mitosis this free duplication (and thus the *pal-1*$^+$ gene) is lost spontaneously at a low frequency, creating clones of *pal-1*$^-$ cells in otherwise *pal-1*$^+$ animals. Using the previously described markers *unc-36*, *dpy-17* and *ncl-1* (refs 4 and 5; E. Hedgecock, personal communication) as lineage markers, we could identify mosaic animals in which the *pal-1* genotypes of V6 and T were inferred to be different (Fig. 1). In class I mosaics T had a mutant *pal-1*$^-$ genotype, whereas V6 had a *pal-1*$^+$ genotype. In all these animals V6 developed normally (that is, it produced ray lineages). This result indicates that *pal-1*$^+$ activity is not required in T. In class II mosaics V6 had a mutant *pal-1*$^-$ genotype, whereas T had a *pal-1*$^+$ genotype. In all of these animals, V6 had a mutant Pal-1 phenotype (produced alae lineages). This result indicates that *pal-1* acts in V6 or in close lineal relatives of V6 that were also mutant in class II mosaics. Because none of these relatives of V6 is located in the posterior body region, it seems unlikely that *pal-1* functions in these cells. Thus we believe that *pal-1*$^+$ acts in the responding cell, V6, to prevent effective cell-cell communication.

This mosaic analysis indicates that T sends a signal to V6 in the wild type, even though V6 cannot respond to it. Thus, in this system, spatial information is not imparted by targeting alae-inducing signals to one type of precursor cell. Rather, alae and ray precursors are both exposed to the signals, and the pattern is established by regulating the cell's ability to respond.

```
   1: .CA AGT TTG AGA ATG TCG GTC GAT GTC AAG TCG GAT TTT TCG GAA AAC GAG TCC TCA TCA ACA CCT TCA CCA ACA
    : ... ser leu arg met ser val asp val lys ser asp phe ser glu asn glu ser ser ser thr pro ser pro thr

  76: ACA GTT CCA GCA GAT GTC ACG TGG CCT CAC TAT CCA ATG ATG CCA TTC ATG CAA CCA CAT CCT CTG AGA GAA AAG
    : thr val pro ala asp val thr trp pro his tyr pro met met pro phe met gln pro his pro leu arg glu lys
                                                                      ↱
 151: ATG CTG CAA CCA ACA TTT GAT CCA CAA ATC TAT GGA AGG TGG TCA CAA ATG GGT GAT ACT GGT TTT TAC GGA CAT
    : met leu gln pro thr phe asp pro gln ile tyr gly arg trp ser gln met gly asp thr gly phe tyr gly his

 226: CCG GAT CTC TAT CCA TTC GGC CTT CCA CAA CTC GCT GCC AAC GGA CAA ATT CCA GCA GTA GAA GCC GTT GAT GTG
    : pro asp leu tyr pro phe gly leu pro gln leu ala ala asn gly gln ile pro ala val glu ala val asp val

 301: AAG CCA CCG CTT TCG AAT GGA AGT AGC AGT AGT GAT AGT GGA ATG TAT CCA TCA CCG AGT GAT ATG ATG ACA CCG
    : lys pro pro leu ser asn gly ser ser ser ser asp ser gly met tyr pro ser pro ser asp met met thr pro

 376: TTC CCA TCA ACA TCA TCT GGA GCT GCA TCT TCT TCA GAG CTT TCA GCA GCT GCT GCT GCT GCA GCC AAC TAC CAG
    : phe pro ser thr ser ser gly ala ala ser ser ser glu leu ser ala ala ala ala ala ala ala asn tyr gln

 451: ATG AGA GCT GCC ACG TGT TAT CAG CAA TCT GTT TGG CCG TTT ATG GAT TAT CAA CAG TTT CAG GGA TTC TCG TGG
    : met arg ala ala thr cys tyr gln gln ser val trp pro phe met asp tyr gln gln phe gln gly phe ser trp

 526: AAA ATG CCT CTT GGA AAC AAT CAT GGA AAG GAT CGA AGA TCA AGC AGT GAT GGG AAA ACA TTA CCA ACG GGA CCT
    : lys met pro leu gly asn asn his gly lys asp arg arg ser ser ser asp gly lys thr leu pro thr gly pro

 601: GGA ACG AAT AAT GTC CGT GTA CGA ACA GCT GAC AAA TAC CGT ATG GTT TAC TCG GAT TAT CAA CGC CTT GAA CTG
    : gly thr asn asn val arg val arg thr ala asp lys tyr arg met val tyr ser asp tyr gln arg leu glu leu

 676: GAA AAA GAA TTC CAT ACA TCG CCA TTC ATA ACA TCA GAT CGC AAG TCC CAA TTG TCG ACG ATG TTA AGC TTG ACA
    : glu lys glu phe his thr ser pro phe ile thr ser asp arg lys ser gln leu ser thr met leu ser leu thr

 751: GAA CGA CAA ATC AAG ATT TGG TTT CAA AAT AGG CGT GCA AAG GAT CGT CGT GAC AAA CAG AAG ATT CGG CTA TAA
    : glu arg gln ile lys ile trp phe gln asn arg arg ala lys asp arg arg asp lys gln lys ile arg leu ***

 826: GTA CTC ATC TAC TTA CAA AGA AAA TGT ACA AAA ATT CTG GGA ATT TTC ATA TTT TTC TAG AAT ATT GTA CAC ATT

 901: TAT TAT ATT TTT ACA TTT CTC TCG CTT GAA TAC GGT TAA ATT CAC ACT TTT ATA TAA TTT CTT CCT GTC CCA TCT

 976: CCC ATT CTC TCA CAT CAG AAT ATT CTA ATC ATC ACT GCC CTT AAC ATA TAT ATA TAT ATA TTT ATA ACT TTC CGA

1051: TCA TTC CTT CTG CTT TGT AAA CTA ATC CAA TCA TTC TCT AAT GAA TTA TTC CCT AAT ATA GAG CTT CTT TAT TTA

1126: TTG TGA TTT CTT GTT TCT TAC CCT TCT CAA GGT TTA GGA AAA AGT TTA GGT CCC CAT TTG TTA TGT ATT TAC TTC

1201: CCA CTC TGA ACG GAT CCA TTT TGA ACA TGT TTT CTA TTT TTT GGA AAA GTT TAG AGA ATT CTT TGC TTT TCT CAC

1276: ATA TCT TGA TTG CAT ATT TTT TAT AAC AAA ACA ATG AAA CAA TTT TGA AGA AT...poly-A
```

FIG. 2 The nucleotide and deduced amino-acid sequence of a *pal-1* cDNA. The arrow indicates the AUG most likely to be the translation start site on the basis of sequences of other *C. elegans* start sites (M. Perry, G. Hertz and W. Wood, personal communication). The homeodomain is underlined. The homeodomain is most similar to the homeodomains of the *Drosophila* gene *caudal* and the mouse gene *cdx-1*. The *pal-1* homeodomain was named *ceh-3* by Burglin *et al.*, who first reported these homologies[11]. The significance of these homologies is unclear. We have found a region of homology between *pal-1* and *mab-5* in addition to the homeodomain. In both genes there is encoded a serine-rich region followed by SAAAAAAAN (in single-letter amino-acid code; shown in box). Such polyamino-acid stretches are common in homeodomain proteins, but their function is unknown[12]. That both *pal-1* and *mab-5* act in V6 to promote ray formation makes this homology possibly significant.

METHODS. The *pal-1* gene was identified by transformation rescue as described[13]. The *pal-1* gene maps genetically close to the gene *ced-4* which has been cloned (J. Yuan and H. R. Horvitz, personal communication; H. Madhani, and C.K., unpublished results). Cosmid clones from the region (kindly provided by A. Coulson) were co-injected into *pal-1(e2091)*; *unc-31(e169)* hermaphrodites with a cosmid containing the wild-type *unc-31* gene, and the non-Unc progeny were scored for the Pal phenotype. The cosmid W05E6 fully rescued the Pal-1 phenotype. Burglin *et al.* cloned and sequenced a homeobox, *ceh-3*, which is located on W05E6 (ref. 11). Thinking that *ceh-3* might be in the *pal-1* gene, we cloned successively smaller fragments containing *ceh-3* and eventually obtained a 5.5-kb *PstI-KpnI* clone (pWPK6) which fully rescues the Pal-1 phenotype. (These transformations were done using as cotransformation markers either *unc-31* (R. Hoskins, personal communication) or the plasmid pRF4, which carries the dominant allele of *rol-6, su1000* (J. Kramer and C. Mello, personal communication).) Two independent cDNAs were obtained by hybridization to one of our rescuing clones. The cDNAs were identical in sequence, except that one clone was 7 base pairs longer at the 5′ end of the gene and had three additional bases before the poly(A) tail. The sequence shown is that of the longer clone (sequenced on both strands using Sequenase 2.0). The first 10 bases of the longer cDNA (and the first three bases of the smaller cDNA) correspond to the last bases of one of the 19-base-pair *trans*-spliced leader sequences found at the 5′ end of many *C. elegans* genes[14]. This leader sequence suggests that these cDNAs are, with the exception of the remaining bases of the *trans*-spliced leader, of full length. The genomic clone that rescues *pal-1*, (pWPK6), contains about 1 kb of sequence upstream of the 5′ end of the cDNA and roughly 400 bases downstream of the 3′ end of the cDNA. On several occasions an additional ray was present in animals transformed with pWPK6, presumably caused by overexpression or ectopic expression of *pal-1*. This may be a result of introducing multiple copies of *pal-1* in the transformation experiments, or the removal of a *cis*-acting control element.

We cloned the *pal-1* gene on the basis of marker rescue. We isolated a 5.5-kilobase (kb) genomic fragment that fully rescued the Pal-1 phenotype in DNA transformation experiments (see Fig. 2 legend). Using a genomic fragment as a probe, we isolated two independent complementary DNA clones (1.3 kb). The DNA sequence of one cDNA is shown in Fig. 2. This sequence contains one large open reading frame that is predicted to encode a 208-amino-acid protein containing a homeodomain, a protein domain found in many transcriptional regulators (Fig. 2). Therefore, *pal-1* is likely to act by binding DNA and regulating transcription.

The activity of *pal-1*$^+$ seems to initiate one developmental pathway (ray lineages) by somehow preventing signals from T from initiating an alternative pathway (alae lineages). Two ways in which the *pal-1* transcription factor might accomplish this seem plausible. First, *pal-1* might act directly to inhibit signal transduction; for example, it could repress transcription of a gene encoding any component of the signal transduction pathway. The failure to transduce the signal that induces alae lineages could allow the cells to initiate an alternative pathway leading to ray production. Alternatively, *pal-1* could promote the ray fate more directly in a way that precludes cell signals from having any effect. For example, *pal-1* could directly activate downstream genes that initiate ray lineages or it could repress genes that promote alae lineages. These models are illustrated in Fig. 4.

The genes *mab-5* and *lin-22* are potential downstream targets of the cell signals and of *pal-1*. The *mab-5* gene is a homeotic gene that contains an *Antennapedia*-like homeobox and allows V6 as well as many other epidermal, neural and mesodermal cells in the posterior body region to generate the rays and other structures that characterize that region[4,6]. The gene *lin-22* is necessary for anterior V cells to generate alae rather than ray lineages[7]. On the basis of genetic data, we previously proposed that *mab-5* and *lin-22* function in a genetic switch to control whether alae or ray lineages develop. In the model, in anterior cells, signals from neighbours enhance *lin-22* activity, which in turn inhibits *mab-5* activity and initiates alae lineages. In V6 (or its descendants), *pal-1* activity blocks or overrides the signalling pathway, and this in turn allows *mab-5* activity to inhibit *lin-22* and initiate ray lineages[1].

The idea that *pal-1* activates *mab-5* in the V6 cell is supported by properties of a *mab-5* gain-of-function mutation, *e1751* (ref. 8), which causes transformations opposite to those of *mab-5* null alleles in several tissues. This mutation alters DNA sequences upstream of *mab-5*, and so may lead to inappropriate *mab-5* transcription (S. Salser and C.K., unpublished results). To find out whether this altered *mab-5* gene could initiate ray lineages without *pal-1*$^+$ activity, we constructed the *pal-1(e2091) mab-5(e1751)* double mutant. The *pal-1* V6 mutant phenotype is suppressed (Fig. 3). The fact that the *mab-5* gain-of-function

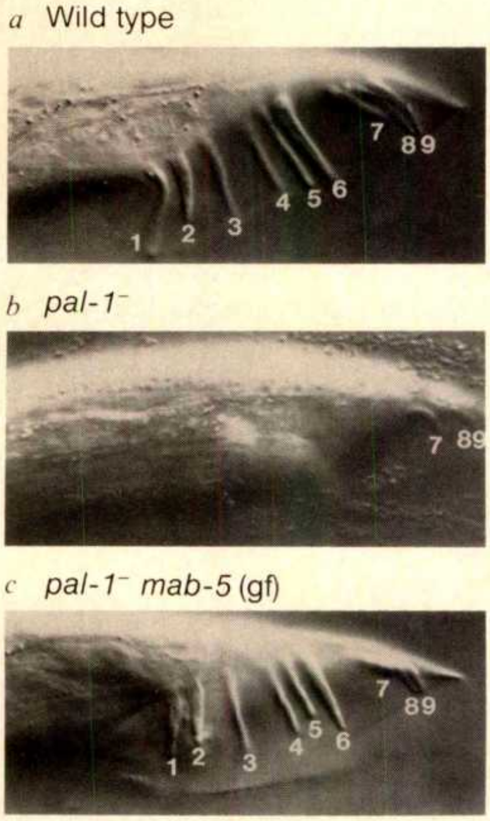


FIG. 3 A *mab-5* gain-of-function mutation overcomes the requirement for *pal-1* activity in V6. *a*, Wild-type male tail, showing the nine sensory rays present on each side of the animal. *b*, Tail of a *pal-1(e2091)*; *him-5(e1490)* male. Only the T-derived rays 7, 8 and 9 are present in the tail. V6 produces alae instead of rays 2–6 in *pal-1(e2091)* mutants. The V5-derived ray 1 is not in the tail because the ray 1 neuroblast does not migrate past the transformed V6 descendants into the tail[1]. *c*, Tail of a *pal-1 mab-5(gf)* male (*unc-79(e1068) pal-1(e2091) mab-5(e1751)*; *him-5(e1490)*). In these animals, the suppression of *pal-1* by *e1751* is virtually complete. On only 5 out of 100 sides were there fewer than the wild-type number of rays, and in those cases V6 made a few rays. By comparison, in *pal-1(e2091)* single mutants, 85% of the sides have no V6-derived rays[1].

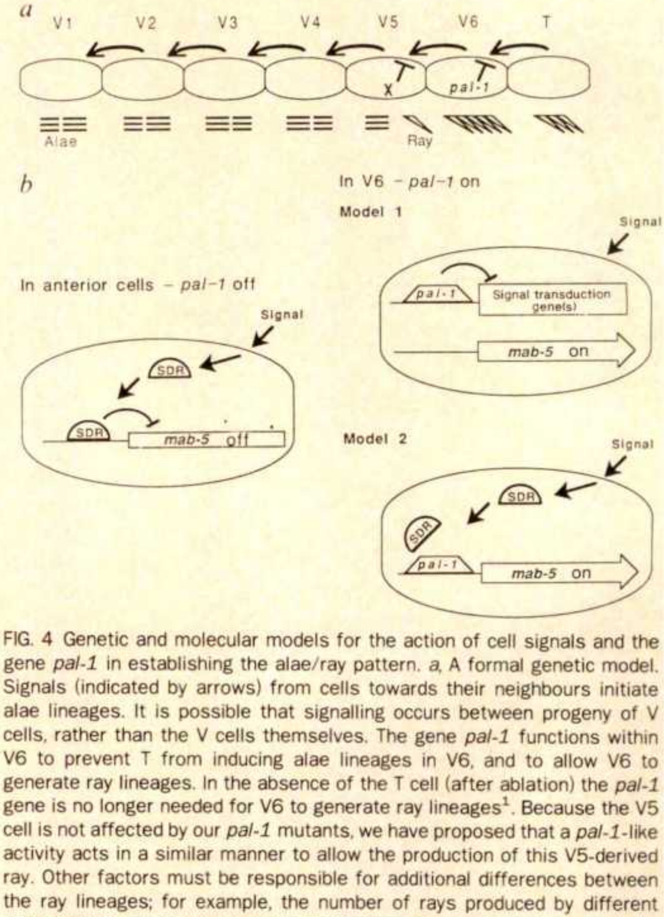


FIG. 4 Genetic and molecular models for the action of cell signals and the gene *pal-1* in establishing the alae/ray pattern. *a*, A formal genetic model. Signals (indicated by arrows) from cells towards their neighbours initiate alae lineages. It is possible that signalling occurs between progeny of V cells, rather than the V cells themselves. The gene *pal-1* functions within V6 to prevent T from inducing alae lineages in V6, and to allow V6 to generate ray lineages. In the absence of the T cell (after ablation) the *pal-1* gene is no longer needed for V6 to generate ray lineages[1]. Because the V5 cell is not affected by our *pal-1* mutants, we have proposed that a *pal-1*-like activity acts in a similar manner to allow the production of this V5-derived ray. Other factors must be responsible for additional differences between the ray lineages; for example, the number of rays produced by different cells. *b*, Two ways in which *pal-1* could function as a transcriptional regulator to change the responsiveness of V6 to intercellular signals. In both models, we suggest that a signal-dependent repressor (SDR) represses transcription of *mab-5* in anterior cells, after being activated in response to intercellular signals by some mechanism. In model 1, wild-type *pal-1* blocks the response to cell signals in V6 (or its descendants) by repressing a gene encoding a component of the signal transduction pathway. In model 2, *pal-1* acts directly on a downstream gene (such as *mab-5* or possibly *lin-22*) to neutralize or override the effects of intercellular signals. Here we show *pal-1* activating *mab-5* transcription by preventing SDR from repressing *mab-5*. The *pal-1* gene could simply prevent SDR from binding, or it might act as a transcriptional activator.

mutation overcomes the requirement for *pal-1*$^+$ function suggests that, in the wild type, the ultimate effect of *pal-1* is to activate *mab-5*. Thus *mab-5* is a candidate for a downstream gene directly regulated by *pal-1*. One way in which *pal-1* could activate a downstream gene such as *mab-5* would be for the *pal-1* protein to bind to the gene in such a way that a second transcriptional regulator—one that is dependent on signals from T—could not function. This would explain why *pal-1* function is required only if the signalling cell T is present (see Fig. 4).

Whatever the means by which *pal-1* activity prevents V6 from responding to intercellular signals, these findings indicate that a mechanism exists for creating unequal signal responsiveness between precursor cells that otherwise have similar developmental potentials. Because this differential responsiveness plays such an important part in producing the pattern of alae and rays, it will be interesting to discover how *pal-1* function can be targeted so precisely to V6. □

Received 3 January; accepted 28 February 1991.

1. Waring, D. A. & Kenyon, C. *Cell* **60**, 123-131 (1990).
2. Sulston, J. & Horvitz, H. R. *Devl. Biol.* **56**, 110-156 (1977).
3. Sulston, J. & White, J. *Devl. Biol.* **78**, 577-597 (1980).
4. Kenyon, C. *Cell* **46**, 477-487 (1986).
5. Austin, J. & Kimble, J. *Cell* **51**, 589-599 (1987).
6. Costa, M., Weir, M., Coulson, A., Sulston, J. & Kenyon, C. *Cell* **55**, 747-756 (1988).
7. Horvitz, H., Sternberg, P., Greenwald, I., Fixsen, W. & Ellis, H. *Cold Spring Harb. Symp. quant. Biol.* **48**, 453-463 (1983).
8. Hedgecock, E., Culotti, J., Hall, D. & Stern, B. *Development* **100**, 365-382 (1987).
9. Sulston, J., Schierenberg, E., White, J. & Thomson, J. *Devl. Biol.* **100**, 64-119 (1983).
10. Rosenbluth, R., Cuddeford, C. & Baillie, D. *Genetics* **109**, 493-511 (1985).
11. Burglin, T. R., Finney, M., Coulson, A. & Ruvkun, G. *Nature* **341**, 239-243 (1989).
12. Scott, M. P. & Carroll, S. B. *Cell* **51**, 689-698 (1987).
13. Fire, A. *EMBO J.* **5**, 2673-2680 (1986).
14. Krause, M. & Hirsh, D. *Cell* **49**, 753-761 (1987).

ACKNOWLEDGEMENTS. We thank H. Madhani for his contribution to the mapping of *pal-1*; members of C.K.'s laboratory and C. Goutte for discussions and comments on the manuscript; C. Martin for the cDNA library; J. Sulston and A. Coulson for cosmid clones; and E. Hedgecock, D. Thierry-Mieg, R. Hoskins and A. Chisolm for sharing unpublished information about *mab-5(e1751)*. Some nematode strains were provided by the *Caenorhabditis* Genetics Center, which is funded by the NIH National Center for Research Resources. This work was supported by the NIH. D.W. was supported by the NIH and the Lucille P. Markey Foundation.

Phosphorylation of two small GTP-binding proteins of the Rab family by p34^{cdc2}

Eric Bailly*, Mary McCaffrey†, Nicolas Touchot‡§, Ahmed Zahraoui‡, Bruno Goud† & Michel Bornens*

* Centre de Génétique Moléculaire, CNRS, 91198 Gif-sur-Yvette, France
† Unité de Génétique Somatique (URA CNRS 361), Institut Pasteur, 75724 Paris, France
‡ Faculté de médecine Lariboisière-Saint Louis, INSERM unité 248, 75010 Paris, France
§ Present address: Department of Biology, Lewis Thomas Laboratory, Princeton University, Princeton, New Jersey 08544, USA

ENTRY of a cell into mitosis induces a series of structural and functional changes including arrest of intracellular transport[1–4]. Knowledge of how the mitotic cycle is driven progressed substantially with the identification of the p34^{cdc2} protein kinase as a subunit of maturation-promoting factor[5–7], the universal regulating component of the mitotic cycle[8]. Activation of the kinase at the onset of mitosis[9] is thought to trigger the important mitotic events by phosphorylating key proteins[10]. Small guanine nucleotide-binding proteins have been implicated in regulating transport pathways. For instance, two small Ras-related GTP-binding proteins, Sec4p and Ypt1p, control distinct stages of the secretory pathway in budding yeast[11–15]. The GTP-binding proteins of the Rab family in rats and humans[16,17] display strong homologies with Sec4p and Ypt1p, and might therefore also be involved in regulating intracellular transport. Indeed, distinct Rab proteins are located in the exocytotic and endocytotic compartments[18–21]. Interruption of vesicular transport during mitosis might involve modification of these proteins. We now present biochemical evidence for a mitosis-specific p34^{cdc2} phosphorylation of Rab1Ap and Rab4p. By contrast, Rab2p and Rab6p are not phosphorylated. We also show that the distribution of Rab1Ap and Rab4p between cytosolic and membrane-bound forms is different in interphase and mitotic cells. This may provide a clue to the mechanism by which phosphorylation could affect membrane traffic during mitosis.

To determine whether the expression of Rab proteins varies during the cell cycle, whole cell extracts from interphase and mitotic HeLa cells were probed with specific polyclonal rabbit antisera raised against human Rab proteins expressed in *Escherichia coli*. The levels of Rab1A, Rab2, Rab4 and Rab6 proteins did not seem to change significantly as cells proceeded from interphase to mitosis (Fig. 1*a*), ruling out any major regulation of the expression of these proteins during the cell cycle. Because the onset of mitosis is known to coincide with a

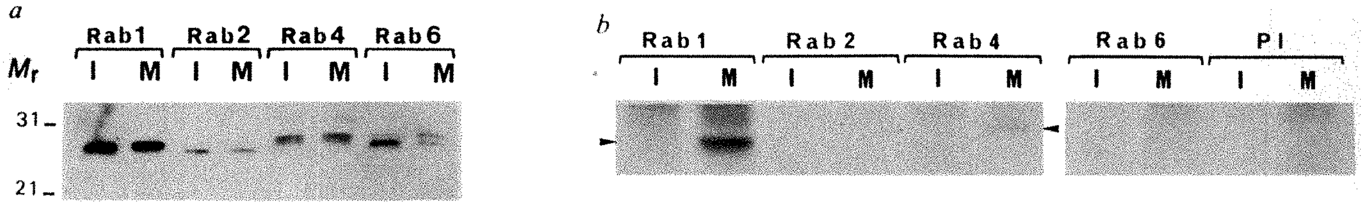


FIG. 1 Immunoblot and ^{32}P-immunoprecipitation analysis of human Rab1A, Rab2, Rab4 and Rab6 proteins during the cell cycle. Cell extracts were prepared from either interphasic (I) or mitotic (M) synchronized HeLa cells. *a*, Comparison of Rab1Ap, Rab2p, Rab4p and Rab6p content in interphasic and mitotic whole HeLa cell extracts. Total proteins (30 μg) were loaded in each lane and probed by western blotting with the corresponding rabbit immune sera. *b*, Immunoprecipitation of Rab proteins from *in vivo* ^{32}P-labelled cell lysates. Preimmune serum (PI) of the rabbit that produced anti-Rab6p was used as a blank. Immunoprecipitates were visualized by autoradiography of SDS gels. Electrophoretic mobilities of bacterially expressed Rab1Ap and Rab4p are indicated by arrowheads.

METHODS. Interphasic HeLa cells were treated with 2.5 mM thymidine for 16 h and then recovered. Cells accumulated in mitosis by a 16-h treatment with 1 μM Nocodazole were collected by pipetting. Characterization of the anti-Rab antibodies has been described elsewhere[18,32]. *a*, Samples were prepared and detected as previously described[33] except that the secondary peroxidase-conjugated antibody was detected with the ECL system according to the manufacturer's instructions (Amersham). *b*, Synchronized cells were incubated for 90 min in phosphate-free RPMI medium containing 7% dialysed FCS before addition of 350 μCi ml^{-1} ^{32}P$_i$ (Amersham). Cells were labelled for 3 h in the continuous presence of the same concentration of thymidine or Nocodazole, and were lysed in modified RIPA buffer (0.1 M Tris-HCl, pH 8.3, 2 mM EDTA, 0.5% Nonidet P-40, 0.5% deoxycholate, 0.1% SDS, 150 mM NaCl, 10 mM NaF, 50 μM sodium orthovanadate, 20 mM β-glycerophosphate, 5 μg ml^{-1} leupeptin/pepstatin, 50 μg ml^{-1} aprotinin and 1 mM PMSF). Immunoprecipitation was performed as described previously[33] and analysed on 12.5% polyacrylamide-SDS gels. After staining with Coomassie blue, gels were dried and exposed to Kodak XA-S films for 72 h at −80 °C with an intensifying screen (Dupont Cronex).

burst of phosphorylation, we then investigated whether these proteins were phosphorylated in M phase. Rab proteins were immunoprecipitated from *in vivo* ^{32}P-labelled interphasic and mitotic cell lysates and analysed by SDS-PAGE and autoradiography (Fig. 1*b*). Whereas Rab2p and Rab6p were not phosphorylated in either interphase or mitosis, ^{32}P-labelled bands comigrating with bacterially produced Rab1Ap and Rab4p were immunoprecipitated from mitotic cell lysates only. The difference in signal intensity between Rab1Ap and Rab4p was in good agreement with the comparative amounts of these proteins as judged by immunodetection (see Fig. 1*a*). These results indicate that Rab1Ap and Rab4p, but not Rab2p and Rab6p, are specifically phosphorylated during mitosis.

We next attempted to identify the protein kinase responsible for the M phase-specific phosphorylation. Examination of the deduced amino-acid sequences[17] showed that only Rab1Ap and Rab4p contained putative consensus motifs for a p34^{cdc2} phosphorylation site in their C-terminal region (Fig. 2*a*). To test whether p34^{cdc2} was the relevant protein kinase, purified Rab1Ap and Rab4p were used as substrates in an *in vitro* phosphorylation assay with immunoprecipitated p34^{cdc2} from either interphase or mitotic cell extracts (Fig. 2*b*). Histone H1 and Rab6p were included as positive and negative controls, respectively. Accordingly, mitotic p34^{cdc2} which displayed a high histone H1 kinase activity was also found to phosphorylate purified Rab1Ap as well as Rab4p but not Rab6p. The prominent phosphorylation of histone H1 compared with that of Rab proteins was expected, as the latter contain one putative site for p34^{cdc2} phosphorylation whereas histone H1 is phosphorylated on multiple sites[22]. Conversely, interphase p34^{cdc2} displayed a very low kinase activity for all exogenous substrates. Similar experiments with the catalytic subunit of the cyclic AMP-dependent type-II protein kinase revealed that none of the Rab proteins tested could be phosphorylated *in vitro* (data not shown).

To obtain further evidence for involvement of p34^{cdc2} in Rab1Ap and Rab4p phosphorylation, we determined whether p34^{cdc2}-depleted mitotic cell extracts could still phosphorylate exogenously added purified Rab proteins. We used the high

a

```
                         * *
Hrab1(184-195) N V K I Q S T P V K - - - - - - - - Q S
Hrab2(180-199) G I K I G P Q H A A T N A T H A G N Q G
Hrab4(180-199) G I Q Y G D A A L R Q L R S P R R T Q A
                                         *
Hrab6(191-200) I K L E K P Q E - - - - - - - - - - Q P
```

b

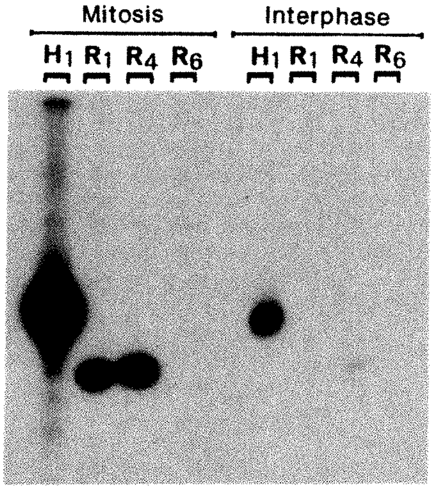


FIG. 2 Rab1Ap and Rab4p are phosphorylated *in vitro* by p34^{cdc2}. *a*, Comparison of the C-terminal amino-acid sequences (single-letter notation) of human Rab1A, Rab2, Rab4 and Rab6 proteins. Localization of p34^{cdc2} consensus sequences in Rab1Ap and Rab4p is indicated by bold letters. Putative phosphorylated residues are marked with an asterisk. Sequences were aligned according to Zahraoui *et al.*[17]. *b*, *In vitro* p34^{cdc2} phosphorylation. Immunoprecipitates of p34^{cdc2} from mitotic and interphase cell lysates were assayed for *in vitro* phosphorylation activity with purified histone H1 (H1), Rab1Ap (R1), Rab4p (R4) and Rab6p (R6) as substrates. Incorporation of radioactive phosphate was followed by SDS-PAGE and autoradiography of the gels.

METHODS. Synchronized HeLa cells were obtained as described in the legend to Fig. 1. Immunoprecipitation of p34^{cdc2} was carried out according to Draetta and Beach[9] with a polyclonal antibody raised against the octopeptide LDNQIKKM corresponding to the C terminus of mammalian-specific cdc2 homologues. *In vitro* phosphorylation of histone H1 (3.3 μg, Boehringer Mannheim) or purified Rab proteins (5 μg) was performed in a 15-μl final volume of TKM buffer (50 mM Tris-HCl, pH 7.4, 25 mM KCl, 5 mM $MgCl_2$ and 0.5 mM DTT) containing 100 μM cold ATP and 1.5 μCi [γ-^{32}P]ATP (Amersham, 5,000 Ci mmol^{-1}). After a 15-min incubation at 37 °C the reaction was stopped by addition of 15 μl of 2 × SDS sample buffer. Aliquots (5 μl) were electrophoresed on 12% SDS-PAGE minigels. After staining with Coomassie blue, gels were dried and autoradiographed at −80 °C for 24 h with an intensifying screen.

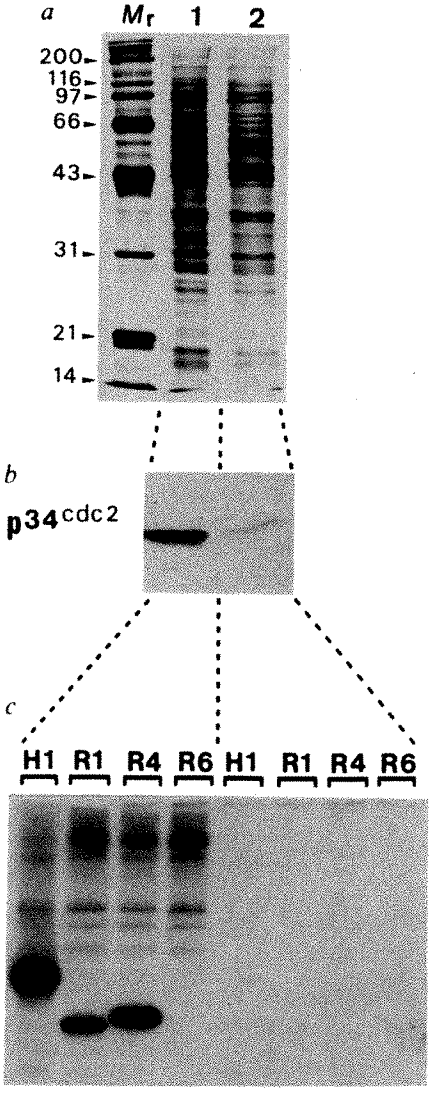


FIG. 3 Depletion of mitotic extracts of p34^{cdc2} abolishes phosphorylation of Rab1Ap and Rab4p. Aliquots of mitotic extracts preincubated either with control Sepharose beads (lane 1) or with p13^{suc1}-carrying Sepharose beads (lane 2) were analysed in the following way. *a*, Protein patterns of both extracts after electrophoresis on a 10% polyacrylamide-SDS gel and silver-staining; M_r, relative molecular mass. *b*, Amount of p34^{cdc2} in both extracts as revealed by western blotting. *c*, Kinase activity of both extracts on exogenous substrates: H1, histone H1; R1, Rab1Ap; R4, Rab4p and R6, Rab6p. The gel was exposed for 24 h at −80 °C with an intensifying screen.

METHODS. The same mitotic extracts as described in Fig. 1 were depleted of endogenous p34^{cdc2} by two successive incubations with p13^{suc1} beads prepared as detailed elsewhere[29]. Bead supernatants were then analysed either by SDS-PAGE electrophoresis followed by silver-staining of the gel or by immunodetection of p34^{cdc2} with the PSTAIR antibody[32]. The phosphorylation assay was carried out as described in the legend to Fig. 2.

affinity of p34^{cdc2} for the fission yeast *suc1*$^{+}$ gene product, p13^{suc1} (ref. 5). After incubation with p13^{suc1} or control Sepharose beads, the cell lysate was recovered and tested for both p34^{cdc2} content and kinase activity. Depletion of p34^{cdc2} in mitotic cell lysates abolished histone H1 as well as Rab1Ap and Rab4p phosphorylation (Fig. 3). In agreement with the results presented in Fig. 2*b*, both control and p34^{cdc2}-depleted interphase extracts exhibited very low kinase activities for exogenous Rab proteins (data not shown). These *in vitro* experiments clearly indicate that p34^{cdc2} is the mitotic protein kinase that phosphorylates Rab1Ap and Rab4p.

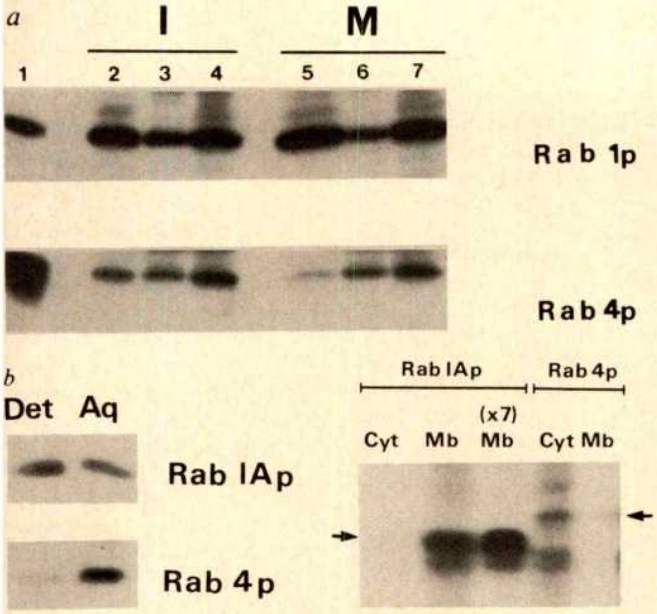


FIG. 4 Partitioning of Rab1Ap and Rab4p. *a*, Immunoblot analysis and measurement of membrane-bound and cytosolic forms of Rab1Ap and Rab4p in interphasic and mitotic cells. The following samples were analysed by immunoblotting with anti-Rab1Ap and anti-Rab4p antibodies: 2, 3, 4, extracts from interphasic cells (I), 5, 6, 7, extracts from mitotic cells (M). Lanes 4 and 7, post-nuclear supernatants; lanes 3 and 6, high-speed supernatants (cytosol); lanes 2 and 5, high-speed pellets (membranes). In lane 1, the samples were 50 ng purified Rab1Ap or Rab4p. The autoradiograph was exposed for 3.5 h at −80 °C. The percentage of total cellular Rab1Ap and Rab4p present in the cytosol of interphase and mitotic extracts was:

	Interphase	Mitosis
Rab1Ap	31 ± 6	19 ± 4
Rab4p	48 ± 5	74 ± 3

The indicated values are the mean ±s.d. of two to four independent experiments. *b*, Immunoblot analysis of Rab1Ap and Rab4p in mitotic cytosol extracted with Triton X-114. A high-speed supernatant prepared as described above (lane 6, *a*) from mitotic cells was extracted twice with Triton X-114 (ref. 34) and the resulting detergent (Det) and aqueous (Aq) phases were analysed by immunoblotting. The autoradiograph was exposed for 14 h at −80 °C. *c*, Subcellular distribution of phosphorylated Rab1Ap and Rab4p in mitotic cells. Cytosolic (Cyt) and membrane (Mb) fractions, prepared as described above, from *in vivo* ^{32}P-labelled mitotic cells were used for immunoprecipitation experiments. Immunoprecipitates were made visible by autoradiography of SDS gels. Lane (×7) Mb corresponds to immunoprecipitation of seven times the volume assayed in lane Mb. Arrows on the left and right hand of the figure indicate the position of Rab1Ap and Rab4p, respectively. The autoradiograph was exposed for 96 h at −80 °C. METHODS. Interphase and mitotic HeLa cells were obtained as described in the legend to Fig. 1. *a*, Post-nuclear supernatants (PNS) were prepared as previously described[18] and centrifuged at 150,000*g* for 1 h at 4 °C to generate cytosol and crude membrane fractions. Aliquots of the different fractions were separated on 12% polyacrylamide-SDS gels. The amount of protein loaded onto the gels was standardized to take into account the distribution of total proteins (PNS) between cytosolic and membrane fractions. Immunoblotting and quantitation using ^{125}I-labelled protein A (Amersham) was then performed as previously described[18]. *b*, Cytosol (200 μl) prepared as described above was extracted twice with an equal volume of 10 mM Tris, pH 7.4, 0.05 M sucrose, 0.3 M NaCl, 2% Triton X-114, protease inhibitors (leupeptin, antipain, pepstatin and aprotinin) to 0.1 μg ml^{-1} and PMSF to 1 mM. The resulting aqueous and detergent phases were precipitated with TCA and analysed by immunoblotting. *c*, *In vivo* labelling of mitotic HeLa cells with ^{32}P$_i$ and immunoprecipitation were carried out as described in the legend to Fig. 1*b*. Cytosolic and membrane fractions were prepared as described above and their volume normalized to reflect directly the proportion of Rab proteins in each fraction.

So how might phosphorylation affect the function of Rab1Ap and Rab4p? Results indicate that the GTP-binding abilities of both proteins are not modified after p34^{cdc2} phosphorylation (data not shown). For several small GTP-binding proteins, the C-terminal region is crucial for membrane attachment, an event required for their proper functioning[23-25]. That the consensus phosphorylation sequence resides in the C-terminal region (Fig. 2*a*) prompted us to compare the partitioning of Rab1Ap and Rab4p on membranes and in cytosolic fractions between interphase and mitotic cells. Most of the Rab4p was cytosolic in mitotic cells as opposed to interphase cells where slightly more than half of the Rab4p present partitioned in the membrane fraction. In contrast, a slight but reproducible reduction of Rab1Ap was detected in the mitotic cytosol (Fig. 4*a*). We were aware of the possibility of artefactual observations in partitioning, due to membrane fragmentation during mitosis. Our results argue against this possibility, however, as the Rab1Ap and Rab4p partitioning trends are in opposing senses in mitotic extracts. Also, the partitioning of Rab2p and Rab6p does not change significantly between interphase and mitosis (data not shown). To address this question more directly, the cytosol from mitotic cells was extracted with Triton X-114. Most of the cytosolic form of Rab4p partitioned into the aqueous phase, again strongly arguing against membrane contamination (Fig. 4*b*). For Rab1Ap, a significant amount of cytosolic protein partitioned in the detergent phase, an observation which has a precedent in the case of Rab3p (ref. 26). To uncover further evidence for the relevance of Rab4p phosphorylation to its partitioning in mitosis we studied the distribution of the mitotically phosphorylated protein between membranes and cytosol. As expected, most of the phosphorylated Rab1Ap and Rab4p were found in the membrane and cytosolic fractions, respectively (Fig. 4*c*).

Our study establishes that both Rab1Ap and Rab4p are phosphorylated *in vitro* and *in vivo* by the M phase-inducing protein kinase p34^{cdc2}, a result which identifies these membrane-associated proteins as a new class of p34^{cdc2} substrates. Rab1Ap, the mammalian homologue[27] of yeast Ypt1p, probably regulates a transport event between endoplasmic reticulum and the Golgi apparatus and/or the Golgi compartments[15,28]. As in the case of Ypt1p in *Saccharomyces cerevisiae* (ref. 16), Rab1Ap is located in the Golgi region of the cell (N.T. and B.G., unpublished data). The precise localization of Rab4p is presently unknown but recent data indicates that it is associated with early endosomes[29]. Tuomikoski *et al.*[30] have reported that active p34^{cdc2} severely inhibits *in vitro* fusion of endocytotic vesicles. Taken together, these observations suggest that phosphorylation of Rab4p by p34^{cdc2} kinase may be fundamental to the mechanism by which endocytosis is arrested in mitotic cells.

Studies on Sec4p indicate that this protein moves from post-Golgi vesicles to plasma membrane in concert with vesicle transport and then returns to vesicles by way of the cytoplasm[12,23]. On the basis of these results, it has been postulated that vesicular transport is coupled to the cycle of small GTP-binding proteins between a cytosolic and a membrane-bound form[23,31]. Our data indicate that p34^{cdc2} phosphorylation may alter the cyclic process for Rab1Ap and Rab4p by perturbing their partitioning and lead to the arrest of intracellular transport during mitosis. ☐

Received 3 December 1990; accepted 7 March 1991.

1. Warren, G., Featherstone, C., Griffiths, G. & Burke, B. *J. Cell Biol.* **97**, 1623–1628 (1983).
2. Featherstone, C., Griffiths, G. & Warren, G. *J. Cell Biol.* **101**, 2036–2046 (1985).

3. Warren, G. *Trends biochem. Sci.* **10,** 439–443 (1985).
4. Warren, G. *Nature* **342,** 857–858 (1989).
5. Dunphy, W. G., Brizuela, L., Beach, D. & Newport, J. *Cell* **54,** 423–431 (1988).
6. Gautier, J., Norbury, C., Lohka, M., Nurse, P. & Maller, J. *Cell* **54,** 433–439 (1988).
7. Labbé, J.-C., Lee, M., Nurse, P., Picard, A. & Dorée, M. *Nature* **335,** 251–254 (1988).
8. Nurse, P. *Nature* **344,** 503–508 (1990).
9. Draetta, J. & Beach, D. *Cell* **54,** 17–26 (1988).
10. Moreno, S. & Nurse, P. *Cell* **61,** 549–551 (1990).
11. Salminen, A. & Novick, P. *Cell* **49,** 527–538 (1987).
12. Goud, B., Salminen, A., Walworth, N. & Novick, P. *Cell* **53,** 753–768 (1988).
13. Schmitt, H. D., Wagner, P., Pfaff, E. & Gallwitz, D. *Cell* **47,** 401–412 (1986).
14. Segev, N., Mulholland, J. & Bostein, D. *Cell* **52,** 915–924 (1988).
15. Bacon, R. A., Salminen, A., Ruohola, H., Novick, P. & Ferro-Novick, S. *J. Cell Biol.* **109,** 1015–1022 (1989).
16. Touchot, N., Chardin, P. & Tavitian, A. *Proc. natn. Acad. Sci. U.S.A.* **84,** 8210–8214 (1987).
17. Zahraoui, A., Touchot, N., Chardin, P. & Tavitian, A. *J. biol. Chem.* **264,** 12394–12401 (1989).
18. Goud, B., Zahraoui, A., Tavitian, A. & Saraste, J. *Nature* **345,** 553–556 (1990).
19. Chavrier, P., Parton, R. G., Hauri, H. P., Simons, K. & Zerial, M. *Cell* **62,** 317–329 (1990).
20. Mollard, G. F. *et al. Proc. natn. Acad. Sci. U.S.A.* **87,** 1988–1992 (1990).
21. Darchen, F. *et al. Proc. natn. Acad. Sci. U.S.A.* **87,** 5692–5696 (1990).
22. Langan, T. A. *J. biol. Chem.* **257,** 14835–14846 (1982).
23. Walworth, N., Goud, B., Kabcenell, A. & Novick, P. *EMBO J.* **8,** 1685–1693 (1989).
24. Molenaar, C. M. T., Prange, R. & Gallwitz, D. *EMBO J.* **7,** 1167–1177 (1988).
25. Willumsen, B. E., Christensen, A., Hubbert, N. L., Papageorge, A. G. & Lowy, D. R. *Nature* **310,** 583–586 (1984).
26. Fischer v. Mollard, G. *et al. Proc. natn. Acad. Sci. U.S.A.* **87,** 1988–1992 (1990).
27. Haubruck, H., Prange, R., Vorgias, C. & Gallwitz, D. *EMBO J.* **8,** 1427–1432 (1989).
28. Baker, D., Wuestehube, L., Scheckman, R., Bostein, D. & Segev, N. *Proc. natn. Acad. Sci. U.S.A.* **87,** 355–359 (1990).
29. Van der Sluijs, P. *et al. Proc. natn. Acad. Sci. U.S.A.* (in the press).
30. Tuomikoski, T., Felix, M. A., Dorée, M. & Gruenberg, J. *Nature* **342,** 942–945 (1989).
31. Bourne, H. *Cell* **53,** 669–671 (1988).
32. Maridonneau-Parini, I., Yang, C. Z., Bornens, M. & Goud, B. *J. clin. Invest.* **87,** 901–907 (1991).
33. Bailly, E., Dorée, M., Nurse, P. & Bornens, M. *EMBO J.* **8,** 3985–3995 (1989).
34. Bordier, C. *J. biol. Chem.* **256,** 1604–1607 (1981).

ACKNOWLEDGEMENTS. We thank J. Hayles, C. Norbury and P. Nurse for the gift of the anti-p34^{cdc2} antibody and M. Dorée and J-C. Cavadore for providing us with p13^{suc1} beads and PSTAIR antibody. We also thank G. Buttin for support and R. M. Rios Sanchez for helpful discussions, I. Gaspard and D. Meur for artwork and S. Brown for proofreading. This work was supported by the CNRS, the Université Pierre et Marie Curie, the Ligue Nationale Française Contre le Cancer (E.B.) and the European Community (M.McC.).

Inhibition of telomerase by G-quartet DNA structures

Alan M. Zahler*, James R. Williamson*, Thomas R. Cech & David M. Prescott

Department of Molecular, Cellular and Developmental Biology,
Department of Chemistry and Biochemistry,
Howard Hughes Medical Institute, University of Colorado,
Boulder, Colorado 80309-0215, USA

THE ends or telomeres of the linear chromosomes of eukaryotes are composed of tandem repeats of short DNA sequences, one strand being rich in guanine (G strand) and the complementary strand in cytosine[1,2]. Telomere synthesis involves the addition of telomeric repeats to the G strand by telomere terminal transferase (telomerase)[3–6]. Telomeric G-strand DNAs from a variety of organisms adopt compact structures[7], the most stable of which is explained by the formation of G-quartets[8,9]. Here we investigate the capacity of the different folded forms of telomeric DNA to serve as primers for the *Oxytricha nova* telomerase *in vitro*. Formation of the K$^+$-stabilized G-quartet structure in a primer inhibits its use by telomerase. Furthermore, the octanucleotide T_4G_4, which does not fold, is a better primer than $(T_4G_4)_2$, which can form a foldback structure[7–10]. We conclude that telomerase does not require any folding of its DNA primer. Folding of telomeric DNA into G-quartet structures seems to influence the extent of telomere elongation *in vitro* and might therefore act as a negative regulator of elongation *in vivo*.

The role of telomerase in telomere extension *in vivo* has been established in *Tetrahymena*[11]. Analogous activity in *Oxytricha*, *Euplotes* and human cells suggests that the function of this enzyme in telomere replication is general[12–14]. In this study we use the species *Oxytricha nova*, whose unusually large number of macronuclear DNA molecules (~10^8 per cell) has facilitated studies of telomeres and telomerase[15].

The G-quartet is a square planar, hydrogen-bonded array of four guanine bases, each of which is both the donor and acceptor of a Hoogsteen base pair[16]. For a telomeric G strand to be intramolecularly folded into a G-quartet, it should have four telomeric repeats[8,9]. The structure is stabilized by physiological concentrations of K$^+$ and, to a lesser extent, Na$^+$ (refs 8, 9 and 17). Two-repeat molecules such as $(T_4G_4)_2$ also form compact intramolecular structures[7–10] but do not show cation-specific stabilization characteristic of G-quartets[8].

We synthesized a series of oligonucleotides oxy-*n* ($n = 1$–8), where *n* is the number of repeats of the *Oxytricha* telomeric DNA sequence d(TTTTGGGG). The mobility of these oligonucleotides in gels containing Tris-borate buffer depends monotonically on their length (Fig. 1), but when 20 mM Na$^+$ or 20 mM K$^+$ is included there is a discontinuity in the relative mobilities of oxy-3 and oxy-4, indicating that oxy-4 is more compact than oxy-3. Oxy-8 also forms a species more compact than oxy-7. These data show that each Na$^+$- or K$^+$-induced intramolecular folding event requires four repeats of single-stranded telomeric DNA.

The activity of the *Oxytricha* telomerase was tested using these same oligonucleotides as primers in the presence and absence of monovalent cations. All reactions contained 10 mM $MgCl_2$ because Mg^{2+} is an absolute requirement for telomerase. The efficiency of extension of each oligonucleotide primer was comparable in the absence of added monovalent cations and in the presence of 20 mM Na$^+$ (Fig. 2*a*), but when 20 mM K$^+$ was included, oxy-4 was far less effective as a primer (lane K of set 4). The activity of oxy-5 and oxy-8 was markedly reduced in K$^+$, although not as much as that of oxy-4; oxy-6 and oxy-7 activities were only slightly reduced.

There is no inhibition by 20 mM Na$^+$ or K$^+$ on telomerase itself, as the activity of primers with $n < 4$ was unaffected by the presence of these ions (Fig. 2*a*). Contamination of primer samples or the possible inhibition of telomerase by the longer primers was discounted by experiments in which primers were mixed together (data not shown). In similar experiments using oligonucleotides composed of telomeric repeats from *Tetrahymena*, extension of tet-2 ($d(TTGGGG)_2$) was not affected by monovalent cations, whereas extension of tet-4 ($d(TTGGGG)_4$) was reduced compared with tet-2, and greatly reduced in the presence of K$^+$ (data not shown).

Oxytricha telomerase is somewhat processive *in vitro*[18], so K$^+$ could affect primer extension at either the initiation or the elongation step. Omitting dGTP from the reaction causes elongation to terminate after addition of the first four T residues. Addition of K$^+$ reduced utilization of oxy-4 but not of oxy-2 (Fig. 2*b*). Therefore the effect of the structure of a primer on its use by telomerase can be attributed at least in part to the initiation step.

When two different substrates at equal concentrations compete for the same enzyme, their relative rates of reaction are determined by their k_{cat}/K_m values; this conclusion holds at all substrate concentrations[19]. An equimolar mixture of oxy-1 and oxy-2 was incubated with telomerase using TTP as the only nucleotide. Oxy-1 reacted about twice as fast as oxy-2 whether or not Na$^+$ or K$^+$ was present (data not shown). Thus oxy-1 has a slightly greater value of k_{cat}/K_m, the rate constant that measures the reaction of free enzyme and free substrate. Because oxy-1 does not form intramolecular structures under these conditions and forms a small amount of intermolecular structure only in K$^+$ (Fig. 1), but was equally active as a primer in the absence and presence of K$^+$ and Na$^+$, we conclude that unstructured primers can be efficiently recognized and extended by telomerase. Confirmation and elaboration of this limited kinetic analysis awaits purification of the enzyme.

Besides the specific monovalent cation effect on the extension

---

* Present addresses: Fred Hutchinson Cancer Research Center, Seattle, Washington 98104, USA (A.M.Z.); and Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA (J.R.W.).

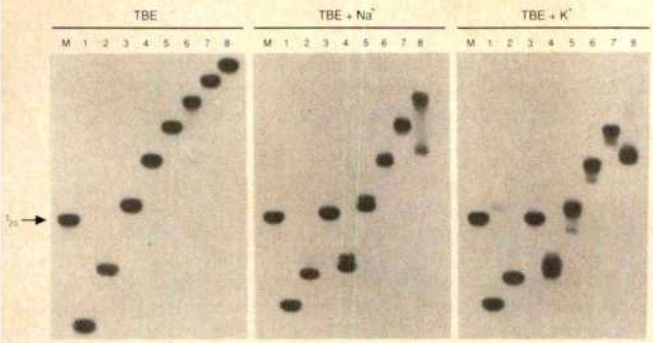


FIG. 1 Folding of *Oxytricha* telomeric oligonucleotides. Single-stranded oligonucleotides composed of $n=1$–8 repeats of the *Oxytricha* telomeric sequence d(TTTTGGGG)$_n$ (oxy-1 to oxy-8, lanes 1–8 in each panel) were subjected to gel electrophoresis under non-denaturing conditions in Tris-borate-EDTA buffer, pH 8.3 (TBE), or in TBE with added Na$^+$ or K$^+$. The unstructured oligonucleotide dT$_{20}$ was run as a marker (lane M). Native gels were run as described[8], except that the temperature was maintained at 15 °C and NaCl or KCl as added to 20 mM.

of oxy-4, oxy-4 was a much poorer primer than oxy-2 even when only Mg^{2+} was present (Fig. 2*a* and *b*). Under all conditions the extension of oxy-4, 5, 6, 7 and 8 was much less than that of oxy-1, 2 and 3. As discussed later, oxy-4 folds in the presence of Mg^{2+} alone, which can explain the reduced efficacy of primers with more than three repeats.

Although telomerase has to be assayed in magnesium-containing buffers, the effect of Mg^{2+} on the structure of *Oxytricha* telomeric DNA had not been previously tested. Dimethyl sulphate treatment of oxy-4 at 15 °C in the presence of 10 mM Mg^{2+}, 10 mM Mg^{2+} with 20 mM Na$^+$, and 10 mM Mg^{2+} with 20 mM K$^+$, all gave methylation-protection patterns similar to that for K$^+$ alone[8], namely patterns indicative of G-quartet formation (data not shown). Also, the ultraviolet-induced cross-link that is characteristic of the folded form of oxy-4 (ref. 8) was observed under all three sets of ionic conditions (data not shown). Therefore in all our telomerase assays the equilibrium for oxy-4 is shifted toward the folded form. The K$^+$ forms of some telomeric DNAs are much more stable than the Na$^+$ forms (ref. 8; I. Tinoco Jr and C. C. Hardin, personal communication), so we investigated whether this difference in stability could explain the effect of K$^+$ in preventing utilization of oxy-4 by telomerase.

Thermal denaturation of oxy-4 indicates that it undergoes a cooperative unfolding transition under all of our ionic conditions (Fig. 3*a*). The T_m for the Mg^{2+}/K$^+$ form is 30 °C higher than for the Mg^{2+}/Na$^+$ form, and 40 °C higher than for the Mg^{2+} form. The Mg^{2+}/K$^+$ form of oxy-4 is calculated to be ~8 kcal mol^{-1} more stable than the Mg^{2+}/Na$^+$ form at 15 °C (Fig. 3*b*). The hypochromicities for the oxy-4 forms under the three sets of ionic conditions are very different (Fig. 3*b*), as are their absorption spectra (Fig. 3*c* and *d*). Although the dimethyl sulphate protection assay showed that the three forms are fundamentally similar, their absorption spectra and hypochromicities indicate that their folded structures differ in detail.

We conclude that the most stable folded form of telomeric DNA, which we propose is a G-quartet structure, is not an obligatory primer for telomerase, and probably cannot serve as a primer at all. Furthermore, there is apparently no secondary structure requirement for the primer, as the oxy-1 octanucleotide is an effective primer. These results do not support an earlier suggestion that telomerase recognizes a folded form of single-stranded DNA[4,7], although there could be differences between the *Tetrahymena* and *Oxytricha* enzymes. But they are consistent with the observation that inosine substitutions, which destabilize G-quartet formation, do not prevent telomerase activity with the *Tetrahymena* enzyme[20]. The telomere-binding protein from *Oxytricha*, a protein that caps the DNA termini and has no known enzyme activity, also selectively binds to the unfolded form of oxy-4 rather than to the most stable folded form[21].

A model for the action of telomerase consisting of sequential binding, polymerization, and translocation steps has been proposed by Blackburn and colleagues[5,6]. The effect of primer structure on telomerase activity can now be incorporated into this model (Fig. 4). For primers with four or more repeats, the concentration of unfolded DNA competent to bind telomerase

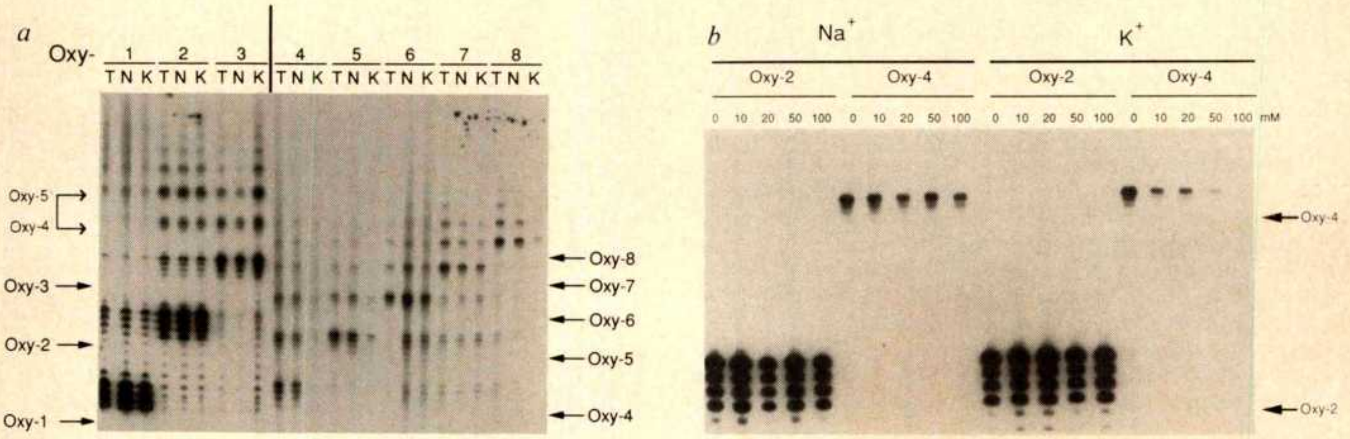
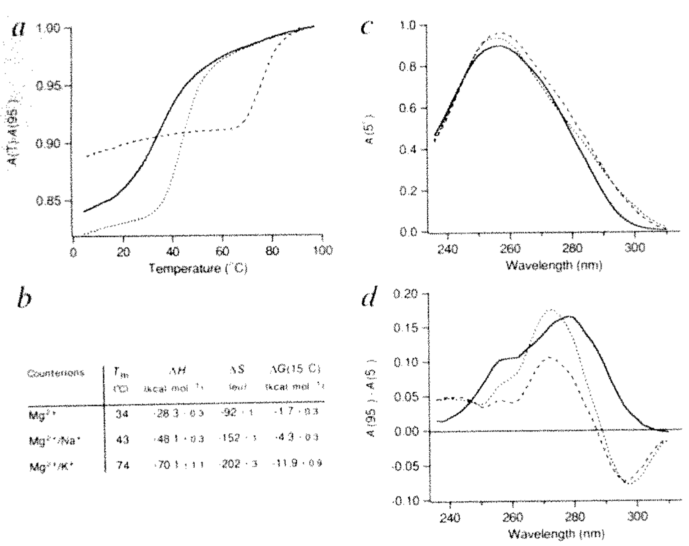


FIG. 2 Effect of Na$^+$ and K$^+$ on telomerase activity using telomeric oligonucleotides as primers. *a*, Standard telomerase assay on oxy-1 to oxy-8 (lane sets 1–8) in Tris-HCl and Mg^{2+} (T lanes), or with Na$^+$ (N lanes) or K$^+$ (K lanes) added to 20 mM. Only elongated products are labelled, and the approximate position of the input unlabelled DNA is indicated at the side for each oligonucleotide. *b*, Initiation of primer elongation by the *Oxytricha* telomerase. Omission of dGTP from the standard telomerase assay results in addition of no more than four T residues to the primer, providing a reasonable approximation to the rate of initiation of telomeric DNA synthesis. The amount of initiation with oxy-2 as primer was unaffected by the addition of Na$^+$ or K$^+$. The amount of initiation with oxy-4 as primer was unaffected by the addition of Na$^+$, but substantial reduction was caused by the addition of K$^+$. Positions of input unlabelled primers are indicated at the side.

METHODS. *Oxytricha nova* was cultivated and its macronuclei isolated as described[12]. Purified macronuclear pellets were suspended in 10 mM Tris-HCl, pH 8.0, 10 mM MgCl$_2$, 8% w/v sucrose at a concentration of 4×10^6 macronuclei per ml and stored in aliquots at −70 °C. Primer oligonucleotides at 0.5–1.0 μM were prepared in 20 mM Na$^+$ or K$^+$, unless otherwise indicated, in 10 μl. Samples were boiled for 5 min and then incubated for 1 h at 15 °C. Telomerase assay mixes were prepared on ice in 40 μl containing 10 μl 4 × buffer (200 mM Tris-HCl, pH 7.5, 40 mM MgCl$_2$), NaCl and KCl as indicated, 1 μl [α-^{32}P]TTP (3,000 Ci mmol^{-1}, 10 mCi ml^{-1}; NEN), 1 μl 25 μM TTP, 1 μl 5 mM dGTP, and 10μl macronuclear extract. Preincubated oligonucleotide (1 μl) was added and the reaction mixture incubated at 15 °C for 30 min. Reactions were stopped by addition of 100 μl 10 mM Tris-HCl, pH 8.0, 25 mM EDTA, 2 μl 25 mg ml^{-1} yeast transfer RNA, 15 μl 5M ammonium acetate and 0.5 ml 100% ethanol. Gel electrophoresis and autoradiography were as described[12].



Counterions	T_m (°C)	ΔH (kcal mol^{-1})	ΔS (eu)	ΔG(15 °C) (kcal mol^{-1})
Mg^{2+}	34	-28.3 ± 0.5	-92 ± 1	-1.7 ± 0.3
Mg^{2+}/Na^+	43	-48.1 ± 0.3	-152 ± 1	-4.3 ± 0.3
Mg^{2+}/K^+	74	-70.1 ± 1.1	-202 ± 3	-11.9 ± 0.9

FIG. 3 Thermal denaturation and spectral analysis of oxy-4 in the presence and absence of Na^+ or K^+. *a*, Thermal denaturation of oxy-4 was monitored by the absorbance (*A*) at 270 nm in buffer containing 10 mM Mg^{2+}(solid line), 10 mM Mg^{2+} plus 20 mM Na^+ (dotted line), and 10 mM Mg^{2+} plu 20 mM K^+ (dashed line). Absorbance was normalized to the absorbance 95 °C. *b*, Thermodynamic parameters ΔH and ΔS were obtained by leas squares fitting of two independent data determinations, such as that show in *a*, to a two-state transition with sloping initial and final baselines. Th Mg^{2+}/Na^+ melting profile was independent of DNA concentration. The lov temperature profile for the Mg^{2+}/K^+ form was dependent on DNA concentra tion over an 8-fold range (0.55–4.5 μM). This is consistent with the observe presence of intermolecular species on native gels under similar condition For Mg^{2+}/K^+, only the concentration-independent transition above 40 was used for the data fitting. The reported errors reflect the uncertainty the parameters based on an absolute absorbance error of ±0.001, and not reflect any systematic errors that may be rather larger. *c*, Absorpti spectra of oxy-4 at 5 °C, normalized to their absorbance at 95 °C. T absorption spectra at 95 °C were identical for the three forms, indicati that denaturation was complete at that temperature. The counterions a indicated as in *a*. *d*, Difference absorbance spectra for oxy-4 in the presen of the same counterions as in *a*. Thermal denaturation profiles and absor ance spectra were determined using a Gilford model 2400 spe trophotometer with a 4-cell sample changer, interfaced for data collecti to an IBM personal computer. The buffer was 5 mM Mg^{2+}–MOPS, pH 7. 1 M Mg^{2+}–MOPS stock solution was prepared by stirring MOPS free ac over magnesium oxide overnight and filtering. Samples were degass before thermal denaturation in a Speed-Vac concentrator for 1 min, tran ferred to quartz microcells, and then overlayered with paraffin oil to minimi evaporation. The absolute absorbance of all three samples was ~1.0 270 nm, which corresponds to an oligonucleotide concentraton of ~3 μ

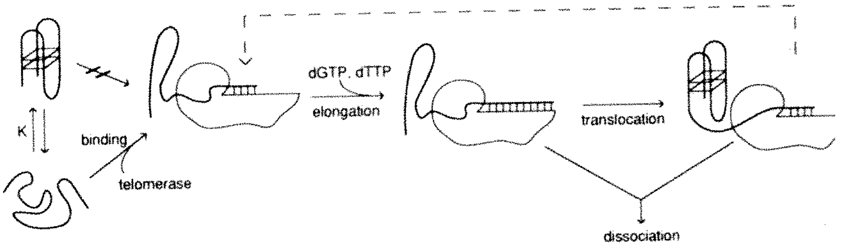


FIG. 4 Model for the effect of primer structure on *Oxytricha* telomerase. The most stable folded form (represented as a G-quartet structure[8]) is unable to bind to the RNA template of telomerase[5,6]. The folded primer is in equilibrium with the unfolded form, which is able to bind the telomerase and is elongated by the addition of dG and T residues. Once the end of the internal RNA template is reached, a translocation step must occur before the next repeat is synthesized[5,6]. If the primer/product becomes sufficiently long (at least 5 repeats for the G-quartet model), the portion of it that is not base-paired to the telomerase RNA template could undergo stable folding. The rate of dissociation of the primer/product from telomerase may be affected by the ability of the primer/product to form such structures. Thus formation of folded structures might contribute the regulation of telomere length *in vivo*.

is reduced because of the pre-equilibrium with the folded form. It is likely that the most stable folded form does not serve as a primer because binding to telomerase requires formation of Watson–Crick base pairs with the enzyme's internal RNA template[5,6,11,13]. The more stable the folded form, the more the pre-equilibrium is shifted to the folded form, and the lower the apparent primer activity. This is consistent with four-repeat molecules being poor primers compared with two-repeat molecules, and with K^+ reducing the activity with four-repeat primers even further. The absence of a large decrease in activity on addition of Na^+ may mean that both the Mg^{2+} and the Mg^{2+}/Na^+ forms of the primer have an unfolded DNA concentration greater than the K_m for primer. But we cannot discount the possibility that the detailed folded structure formed in the presence of Na^+ or Mg^{2+} can be opened by telomerase, whereas that in K^+ cannot.

Formation of a folded structure in the portion of the primer not base-paired to the RNA template might also affect dissociation rates. G-quartet formation by a sufficiently long primer that emerges from the active site could alter the rates of any of the steps in the mechanism. Some evidence for such an effect can be seen in Fig. 2*a*. In the lanes for input primer oxy-1, 2 or 3, there is a discontinuity in the intensities of the products at a length that corresponds to oxy-5. When one repeat is base-paired to the internal template, the four unpaired repeats should still be able to fold into a structure. If G-quartet formation enhances dissociation of the one bound repeat, the five repeat products should be overrepresented relative to the four repeat products, as we observed.

Our results suggest that G-quartets may contribute to regulation of telomere length *in vivo*. This regulation could achieved in either of two ways: by direct inhibition of telomer binding to long telomeric sequences, or by altering the diss ation rate of telomeric primers from telomerase. All kno telomeric sequences are G-rich on the strand that is exten by telomerase, suggesting that the capacity of these sequen to adopt G-quartet structures is not a coincidence.

Received 29 November 1990; accepted 4 March 1991.

1. Blackburn, E. H. & Szostak, J. W. *A. Rev. Biochem.* **53,** 163–194 (1984).
2. Zakian, V. A. *A. Rev. Genet* **23,** 579–604 (1989).
3. Greider, C. W. & Blackburn, E. H. *Cell* **43,** 405–413 (1985).
4. Greider, C. W. & Blackburn, E. H. *Cell* **51,** 887–898 (1987).
5. Greider, C. W. & Blackburn, E. H. *Nature* **337,** 331–337 (1989).
6. Shippen-Lentz, D. & Blackburn, E. H. *Science* **247,** 546–552 (1990).
7. Henderson, E., Hardin, C. C., Wolk, S. K., Tinoco, I. Jr & Blackburn, E. H. *Cell* **51,** 899–908 (1
8. Williamson, J. R., Raghuraman, M. K. & Cech, T. R. *Cell* **59,** 871–880 (1989).
9. Sundquist, W. I. & Klug, A. *Nature* **342,** 825–829 (1989).
10. Sen, D. & Gilbert, W. *Nature* **344,** 410–414 (1990).
11. Yu, G.-L., Bradley, J. D., Attardi, L. D. & Blackburn, E. H. *Nature* **344,** 126–132 (1990).
12. Zahler, A. M. & Prescott, D. M. *Nucleic Acids Res.* **16,** 6953–6972 (1988).
13. Shippen-Lentz, D. & Blackburn, E. H. *Molec. cell. Biol.* **9,** 2761–2764 (1989).
14. Morin, G. B. *Cell* **59,** 521–529 (1989).
15. Klobutcher, L. A. & Prescott, D. M. in *The Molecular Biology of Ciliated Protozoa* (ed. Gall, 111–154 (Academic, Orlando, 1986).
16. Gellert, M., Lipsett, M. N. & Davies, D. R. *Proc. natn. Acad. Sci. U.S.A.* **48,** 2013–2018 (19
17. Oka, Y. & Thomas, C. A. Jr *Nucleic Acids Res.* **15,** 8877–8898 (1987).
18. Zahler, A. M. thesis, Univ. Colorado (1990).
19. Fersht, A. *Enzyme Structure and Mechanism* 2nd edn, 112 (Freeman, New York, 1985).
20. Henderson, E., Moore, M & Malcolm, B. A. *Biochemistry* **29,** 732–737 (1990).
21. Raghuraman, M. K. & Cech, T. R. *Nucleic Acids Res.* **18,** 4543–4552 (1990).

ACKNOWLEDGEMENTS. We thank M. K. Raghuraman for discussions. J.R.W. was a fellow of th Coffin Childs Memorial Fund for Medical Research T.R.C. is Investigator, Howard Hughes M Institute and American Cancer Society Professor. This work was funded by grants from th (D.M.P. and T.R.C.).

Anti-Cancer Drug Design

An international journal covering an important area of common interest for medicinal chemists, biochemists, biophysicists and oncologists

- Concentrates on the rational scientific principles involved in the development of new anti-cancer agents
- Publishes original research papers which reflect the increasing importance of the tools of structural and molecular biology as well as the more traditional methodologies of pharmacology, chemistry and biophysics
- Publishes both experimental and clinically orientated papers
- Listed in **Index Medicus, Excerpta Medica, BIOSIS, Current Contents** and **Chemical Abstracts**
- Now one of the top twenty most intensively cited primary research oncology journals in the world, as monitored by the latest SCI Journal Citation Reports
- Rapid publication of full papers and communications

NEW FOR 1991!

- New American editor, to give added coverage to medicinal chemistry and related areas
- Frequency increased to bi-monthly

Editors

Stephen Neidle
(INSTITUTE OF CANCER RESEARCH, SUTTON, UK)
Editor, UK & Rest of World

Stanley Crooke
(ISIS PHARMACEUTICALS, CALIFORNIA, USA)
Editor, The Americas
Cellular and molecular pharmacology; molecular biology

Laurence Hurley
(UNIVERSITY OF TEXAS AT AUSTIN, USA)
Editor, The Americas
Medicinal chemistry; molecular drug design, physical biochemistry; drug-DNA interactions

Shigeru Tsukagoshi
(CANCER CHEMOTHERAPY CENTER, TOKYO, JAPAN)
Editor, Japan

Editorial Board

F Arcamone, *Italy*
K D Bagshawe, *UK*
J R Brown, *UK*
A H Calvert, *UK*
J S Cohen, *USA*
W A Denny, *New Zealand*
B W Fox, *UK*
K R Harrap, *UK*
S M Hecht, *USA*
D W Henry, *USA*
A J Hopfinger, *USA*
M Jarman, *UK*
J B LePecq, *France*
S J Lippard, *USA*
J W Lown, *Canada*
J A Montgomery, *USA*
V L Narayanan, *USA*
G R Pettit, *USA*
Y Sakurai, *Japan*
A C Sartorelli, *USA*
P Schein, *USA*
D Shugar, *Poland*
M G F Stevens, *UK*
G T Stevenson, *UK*
M J Waring, *UK*
P Workman, *UK*

To subscribe, to request a sample copy, or to obtain Instructions to Authors, please write to:

Dionne Stocking, Macmillan Press Ltd, Houndmills,
Basingstoke RG21 2XS, England

Telephone number: +44 256 29242
Fax number: +44 256 810526

Subscription Information ISSN 0266 9536 Bi-monthly

	Rate for 2 Years	Rate for 1 Year
EEC	£220	£110
Rest of World	£242 £290 (airmail)	£121 £145 (airmail)

We welcome payment in your own currency, converted at the current rate.

M

The first step-by-step guide to human gene mapping

Human Gene Mapping Techniques

By **Helen Donis-Keller, PhD,** Washington University School of Medicine, St. Louis, Missouri

Often a first step towards the understanding of the molecular basis of an inherited disorder is pinpointing the disease causing gene to a chromosome region. Once the chromosome region is identified, experiments to directly clone and characterize the gene can then be undertaken.

Human Gene Mapping Techniques is a step-by-step guide to designing a mapping project, identifying the chromosome location of a gene, developing a high resolution genetic map, and cloning a gene once its chromosome location is known. Detailed laboratory procedures include establishment of permanent cell lines, genetic linkage analysis techniques, physical localization of DNA sequences by *in situ* hybridization, and chromosome walking using yeast artificial chromosome vectors (YACs). This is a practical guide that includes automated laboratory procedures, sources for equipment and reagents, and an extensive list of citations for additional reading. With a major emphasis on genetic linkage mapping, this book presents strategies for mapping genes for researchers who have little acquaintance with genetic mapping but some experience with molecular biology and gene cloning techniques.

Tentative Contents: Meiotic Genetic Linkage Mapping Methods • Radiation Hybrid Genetic Linkage Mapping • Linkage Mapping Using Male Meioses • *In Situ* Hybridization of Cloned DNA to Chromosomes • Somatic Cell Rodent-Human Hybrid Panel Analysis • Construction of Single Chromosome Deletion Panels • Chromosome Sorted Blots for Gene Localization • Data Acquisition, Storage, Access, Communications • Physical Mapping By Pulsed-Field Gels • Yeast Artificial Chromosomes (YAC) Cloning • Constructing Overlapping Clone Maps

December 1990, 250 pp., 0-333-53541-3, £27.95

Send orders to Globe Book Service Ltd., FREEPOST, Stockton House, 1 Melbourne Place, London WC2B 4LF, UK. Or call : 01-379-4687. We accept Access/MasterCard, Visa, Diners' Club and American Express. Please add £2.00 postage and packing. FREEPOST applies to UK only. (Globe book Services is a division of Macmillan, Publishers, Ltd.)

M

LONDON Julie Skeet, 4 Little Essex St, London WC2R 3LF. Telephone: 071 872 0102. Fax: 071 240 2408.
NEW YORK — Marianne Ettisch, 65 Bleecker Street, New York, NY 10012. Telephone: (212) 477 9625. Fax: (212) 505 1364.
PARIS Clare Newell, BP No 804, 75828 Paris, Cedex 17. Tel: (1) 48 87 24 43. Fax: (1) 48 87 46 71.
MUNICH — Sasha Panter, Sandstrasse 41 8000 München 2. Telephone: (089) 52 40 82. Fax: (089) 5 23 22 22.
TOKYO — Phillip Hamill, Shin-Mitsuke Building (4F), 3-6 Ichigaya Tamachi, Shinjuku-ku, Tokyo 162. Telephone: (03) 3267 8751. Fax: (03) 3267 8746.

RATES UK & Rest of world — Display £24 per centimetre. Semi-Display £20 per centimetre. Minimum 3 cm. £3 is charged for a box number.
CONDITIONS The Publisher reserves the right to refuse, amend, withdraw or otherwise deal with all advertisements submitted to him at his absolute discretion and without explanation. The Publishers will not be liable for any loss occasioned by the failure of any advertisement to appear from any cause whatever, nor do they accept liability for printers' errors, although every care is taken to avoid mistakes.

CANCELLATIONS MUST BE RECEIVED NO LATER THAN 5PM ON THURSDAYS PRIOR TO ISSUE DATE.

Washington
WASHINGTON·UNIVERSITY·IN·ST·LOUIS

TENURE TRACK ASSISTANT PROFESSOR

MOLECULAR BIOLOGY AND PHARMACOLOGY

/ashington University School of Medicine is recruiting an ıdividual at the level of Assistant Professor in the tenure track. 'utstanding scientists working within the broad area of molecular iology will be considered with an emphasis on those who have a trong background in mammalian genetics and who are expert at sing transgenic mice to examine fundamental questions in gene ɘgulation and function.

ıterested applicants should forward a curriculum vitae, a hort summary of research plans, and the names and ddresses of three references to: Search Committee, c/o effrey Gordon, Head, Department of Molecular Biology and 'harmacology — Box 8108, Washington University School of ⁄ledicine, 660 South Euclid Avenue, St Louis, MO 63110.

Vashington University is an Affirmative Action/Equal Opportunity :mployer. (NW6641)A

EMBL

The European Molecular Biology Laboratory, an international research organization situated in Heidelberg, Germany invites applications for a

POSTDOCTORAL RESEARCH POSITION

A postdoctoral position is available to study the role of fibrillarin, a nucleolar snoRNA protein, in pre-ribosomal RNA processing and ribosome biogenesis in the yeast system by molecular and genetic approaches. The position is available immediately for a period of two years.

Please send curriculum vitae and names of two referees to: Dr. E.C. Hurt or Dr. D. Tollervey.

(Tel. 0 62 21/38 73 63) (W8421)A

EMBL
Postfach 10.2209
D-6900 Heidelberg
Federal Republic of Germany

Ph.D. POSITIONS

Institute of Obstetrics & Gynaecology, R.P.M.S.

Three positions are available for post-graduate scientists who have, or expect to receive, a life science degree of 2(i) or better. The posts are outlined below, and candidates should contact the relevant supervisor at Queen Charlotte's Hospital or Hammersmith Hospital for more information.

Post 1. MRC Studentship: (Code AG/MRC).
The appointee will work on one of the research projects shown below.

1. Molecular mechanisms of steroid hormone action in human endometrial and breast cancer. (Dr. J. White — HH).
2. The mapping and cloning of a gene causing X-linked cleft palate in an Icelandic family. (Dr. G. Moore — QCH).
3. Interactions between trophoblast, decidua and platelets in the pathogenesis of pre-eclampsia. (Dr. M. Sullivan — HH).
4. Transtrophoblast transfer of zinc in relation to the pathogenesis of abnormal fetal growth. (Dr. D. Bloxam — QCH).

Post 2. Birthright Research Assistantship (Code AG/GEM).
Dr. G. E. Moore & Dr. P. R. Bennett (QCH).
This project will involve the use of molecular biology techniques to investigate prostacyclin synthase gene expression in pregnancy.

Post 3. Birthright Research Assistantship: (AG/MHFS).
Professor M. G. Elder & Dr. M. H. F. Sullivan (HH).
The project is part of a large investigation funded by Birthright into the activation of decidual stromal cells and macrophages in pre-term labour. A post-doctoral scientist has already been appointed. The work will mainly involve tissue culture and RIA techniques.

All these positions will be available from September/October 1991. Candidates should indicate clearly which position or positions they wish to be considered for, since short-listing for each position will take into account the interests of the candidates. Applications by c.v. should be sent to The Personnel Office, R.P.M.S., 150 Du Cane Road, London W12 0NN, and must be received by 14th May 1991, and should include the names, addresses and telephone numbers of two referees.

For further information please contact Dr Sullivan on 081-743 2030 ext. 2377. (6090)A

UNIVERSITY OF BRISTOL

Department of Geology

POSTDOCTORAL RESEARCH ASSISTANT

(Experimental Petrology)

Applications are invited for this 3-year NERC-funded position involving experimental study of noble gas solubility and diffusion in melts, glasses and crystals. Additional experimental and theoretical work related to magma degasing processes will be encouraged. The successful applicant must have PhD completed at time of appointment (Summer, 1991) and previous experience in experimental petrology is highly desirable.

Informal enquiries may be made to Dr M Carroll on 0272 303155, Fax 0272 253385.

For further details telephone Bristol 256450 (ansaphone after 5.00pm) or write to The Personnel Office, Senate House, Bristol BS8 1TH. Please quote reference 656.

An Equal Opportunities Employer. (6085)A

THE UNIVERSITY OF ADELAIDE
South Australia

invites applications from both women and men for the following position:

PROFESSOR OF CROP PROTECTION (Tenurable)

(Ref: 8787) in the DEPARTMENT OF CROP PROTECTION within the Faculty of Agricultural and Natural Resource Sciences, located at the Waite Agricultural Research Institute. Applications are invited for the Chair of Crop Protection which becomes vacant in January 1992 upon the retirement of Professor A Kerr, FRS, FAA.

The Waite Agricultural Research Institute is situated on a delightful campus about 7km from the city centre. General facilities are excellent. Also on the campus are the CSIRO Divisions of Horticulture, Soils and Water Resources, as well as the Australian Wine Research Institute.

The Department of Crop Protection is concerned with research, scholarship and teaching in plant pathology, entomology and weed science, with increasing emphasis on the use of molecular approaches to attacking problems of agricultural importance. The appointee should have distinguished research and teaching interests in one of these areas and provide leadership in teaching and in the further development of an active postgraduate research school.

The Faculty of Agricultural and Natural Resource Sciences is located across two campuses, at the Waite Institute and Roseworthy Agricultural College. The Department of Crop Protection is located at the Waite Campus but provides teaching for students on both campuses.

Further information concerning the Department and duties of the post may be obtained from the Dean of the Faculty, Professor H W Woolhouse (tel. (61 8) 372 2201; Fax (61 8) 338 2744).

It is University policy to encourage women to apply for consideration for appointment to tenurable academic appointments. Holders of full-time tenured or tenurable academic appointments have the opportunity to take leave without pay on a half-time basis for a specific period of up to ten years when this is necessary for the care of children.

INFORMATION about the general conditions of appointment and selection criteria may obtained from the Director, Personnel Services at the University, or from Appointments (39267), Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF.

SALARY per annum: A$67,812.

APPLICATIONS, IN DUPLICATE, quoting reference number 8787 and giving full personal particulars (incuding whether candidates hold Australian permanent residency status), details of academic qualifications and names and addresses of three referees should reach the Director, Personnel Services at the University of Adelaide, GPO 498, Adelaide, South Australia, 5001, Telex UNIVAD AA 89141, Facsimile (61 8) 223 4820 not later than 30 June 1991.

The University reserves the right to make enquiries of any person regarding any candidate's suitability for appointment, not to make an appointment or to appoint by invitation.

THE UNIVERSITY OF ADELAIDE IS AN EQUAL OPPORTUNITY EMPLOYER.

(W8414)A

UNIVERSITY OF NOTTINGHAM
SCHOOL OF AGRICULTURE
Climatic Change and Tropical Crops

A major ODA-funded project will investigate the effects of climatic change on tropical crops in the developing world. Relationships between atmospheric CO_2, temperature, and water stress will be studied using controlled-environment glasshouses. Results will be incorporated into existing crop simulation models. Initially, efforts will concentrate on two key tropical crops, groundnut (peanut) and sorghum.

Applications are invited for 2 post-doctoral research assistants to join the team working on this new project.

Graduates who have, or soon expect to obtain, a PhD in a relevant discipline are encouraged to apply sending a C.V. Ideally, candidates will have a background in one of the following: Physiology, Physics, Mathematics, or Environmental Science.

For both posts the ability to absorb and develop new ideas is a greater requirement than specific experience.

Salary Scales: RA IA £11,400 to £13,495 (Pay award Pending).

For further information write/phone (0602 484848) either Dr Sayed Azam-Ali (x8202) or Dr Neil Crout (x8255), University of Nottingham, School of Agriculture, Sutton Bonington, Loughborough, LE12 5RD.

(6070)A

EMPLOYMENT OPPORTUNITY

INSTRUCTOR (RESEARCH TRACK)

An Instructor (research track — non-tenured) position available to perform studies on the function ar regulation of human neutrophils. Primary responsibiliti center on independent biochemical research on sign transduction pathways and technical instruction ar counselling of graduate students.

The PhD degree in Biochemistry with experience oxidative enzymes and lipid related methodologies a required. Should have technical skills in cell isolatio microscopy, UV-visible spectroscopy, subcellul fractionation, TLC, HPLC, and radioisotope handling.

Salary $27,500 per year and forty (40) hours per week.

Please send resume to the following address: **Georgi Department of Labor, 1275 Clarendon Ave, Avondal Estate, Georgia 30002, or the nearest Georgia Jo Service Center. Ref control # 5476500 EOE.**

(NW6640)A

THE UNIVERSITY OF CALGARY

Assistant Professor in Evolutionary Ecology

The University of Calgary Department of Biological Science invites applications for an Assistant Professor (tenure-track) i Evolutionary Ecology. Applicants should have a stron background in ecological interactions (particularly in plant/anima inter-actions) from an evolutionary perspective and preferabl with a field as opposed to laboratory orientation. Appointmen date 15 July 1991 but a later date can be arranged by mutua consent.

Qualifications include a PhD and post-doctoral experience, commitment to excellence in teaching at the Undergraduate an graduate levels and evidence of a strong and productive researc program. NSERC-University Research Fellows are particular invited to apply. Salary commensurate with qualifications an experience.

In accordance with Canadian immigration requirements priority will be given to Canadian citizens and permanen residents of Canada. The University of Calgary has a Employment Equity Program and encourages application from all qualified candidates, including women, aborigina people, visible minorities, and people with disabilities Applicants should sent a curriculum vitae and have letter sent from three referees before May 30, 1991 to: Dr R. W Davies, Head, Department of Biological Sciences, The University of Calgary, 2500 University Drive NW, Calgary, Alberta, Canada T2N 1N4. Facsimile: (403) 289-9311.

(NW6637)A

SCHOOL OF PHARMACY **UNIVERSITY OF LONDO**
LECTURESHIP IN PHARMACEUTICAL CHEMISTRY

Applications are invited from pharmacists and pharmaceutically-trai chemists or biochemists for a Lectureship in the School with research interest synthetic medicinal chemistry, pharmaceutical analysis, drug struct metabolism, macromolecular structure, drug macromolecular interactions drug delivery and formulation. Candidates trained in other relevant areas also encouraged to apply. Preference will be given to pharmacists.

The successful candidate will be required to teach chemical or biochem sciences on the BPharm and the BSc Toxicology and Pharmacol undergraduate courses.

Salary on scale £12,086-£16,755 (Lecturer A) or £17,455-£22,311 (Lecturer B) p £1,767 London Allowance. For an informal discussion about the post ple contact Professor W A Gibbons (tel: 071 753 5884). For further information ab the School please contact **Mr N J Rampley, Assisant Secretary (071 753 581 the School of Pharmacy, 29-39 Brunswick Square, London WC1N 1AX** whom applications in the form of a curriculum vitae to include a propos programme of research, and naming three referees should be submitted by later than 31 May, 1991.

(6078)A

Cancer Research Campaign

ST GEORGE'S HOSPITAL & INSTITUTE OF CANCER RESEARCH, LONDON AND SURREY

Post-Doctoral Research Fellow in Biochemistry/Pharmacology & Technician

Two posts are available immediately to work on a 5 year joint project to develop Magnetic Resonance Spectroscopy (MRS) for (i) non-invasive study of metabolic changes in tumours in response to chemotherapy; and (ii) the investigation of drug disposition and pharmacokinetics in vivo.

Chemotherapeutic agents developed in the ICR Section of Drug Development will be studied using the 1.9 and 4.7 Tesla laboratory MRS instruments at St. George's and, in clinical trials, on the 1.5 Tesla system at ICR. Both posts will be based in the CRC Biomedical Magnetic Resonance Reseach Group, Department of Cellular and Molecular Sciences, at St George's but will also work in the units in ICR, Sutton.

The Research Fellow will develop this collaborative programme with the support of the technician. He/she will be an experienced post-doctoral scientist with qualifications and a research record in biochemistry, pharmacology or related areas. Experience in MRS would be an advantage. Salary according to age and experience on the RIA scale (to £17,455 + £1,767 London Weighting), plus a non-superannuable CRC enhancement equivalent to two increments on the salary scale, subject to annual review.

The Technician will carry out supporting work such as drug testing, will maintain and passage tumours, and will carry out routine laboratory MRS studies. Experience in drug development or testing, or similar work, will be required. Salary according to age and experience on the TG4 scale (to £11,251 + £1,070 London Weighting).

Further information from Prof. J.R. Griffiths (St George's Hospital) 081 672 9944 ext 55811; Dr I. R. Judson (ICR Drug Development), 081 643 8901 ext 4261; Dr M. O. Leach (ICR MRS), 081 642 6011 ext 3338. Application forms from the Personnel Officer, St George's Hospital Medical School, Crammer Terrace, London SW17 ORE.

INSTITUTE OF CANCER RESEARCH CRC BIOMOLECULAR STRUCTURE UNIT, SURREY

Molecular Modelling of Drug-DNA Interactions

A molecular modeller is sought to work on studies of drug-DNA interactions, especially the design of novel sequence specific agents. Prior experience in an aspect of molecular mechanics/dynamics is preferred. Excellent computational and graphics facilities are available and the work will be in collaboration with established chemical and X-ray structural groups in the Unit.

This position is for three years in the first instance and will be on the grade 2 scale (salary range £13,853 - £18,522 per annum plus a non-superannuable CRC enhancement equivalent to, two increments on the salary scale, subject to annual review). The post is based at Sutton in Surrey and is available immediately.

Informal enquiries may be made to Professor Stephen Neidle (telephone 081 643 8901 ext. 4251), from whom further details are available. Formal applications should be made to The Personnel Officer, Institute of Cancer Research, 17A Onslow Gardens, London, SW7 3AL, with the names and addresses of two referees, quoting reference number 4.91.5.N.3.

Applicants are advised that smoking is prohibited in the Institute's premises.

CRC GRAY LABORATORY, MOUNT VERNON HOSPITAL, NORTHWOOD

Post Doctoral Scientist & Scientific Officer

Two positions are available immediately on a 3 year project grant, funded by the UKCCCR, working with Dr M. C. Joiner and Dr J. E. Arrand at the CRC Gray Laboratory. The project will compare lethal and mutagenic effects of low doses of ionising radiation on human cells in vitro, in order to establish the role of induced repair processes in the radiation response.

The Scientist should ideally have experience (or interest) in radiobiology, tissue culture and molecular biology, although candidates with experience in only one or two of these areas are encouraged to apply. Salary will be £15,262 including London Weighting plus a non-superannuable CRC enhancement equivalent to two increments on the salary scale, subject to annual review.

The Scientific Officer may be appointed at SO or HSO depending on experience. Salary will be up to £13,672 including London Weighting plus a non-superannuable pension supplement.

The CRC Gray Laboratory is set in pleasant rural surroundings in the grounds of Mount Vernon Hospital in the Greater London area. It is one of the leading institutes for radiation research with excellent facilities for multidisciplinary research.

Applicants can telephone us to discuss the project informally on 09274 28611; or send applications, including CV and names and addresses of 3 referees,to : Dr M. C. Joiner, CRC Gray Laboratory, PO Box 100, Mount Vernon Hospital, Northwood, Middlesex, HA6 2JR.

Fighting cancer on all fronts.

(6102)A

The University of Queensland

THE UNIVERSITY OF QUEENSLAND
Brisbane, Australia

RESEARCH FELLOW/SENIOR RESEARCH FELLOW: LIPOPROTEIN SCIENTIST

(Ref No 17991)

Department of Medicine
University of Queensland/Royal Brisbane Hospital

A major joint project is planned to investigate a novel process based on plasma delipidation for the treatment of atherosclerosis. Accordingly, there will be two positions available for scientists with a minimum of a PhD and research experience in lipoprotein metabolism. The positions will be available immediately and the duration is until 31 December 1993. Salary range per annum; Research Fellow A$33,163-A$43,096; Senior Research Fellow A$43,984-A$51,015.

Applicants who are available for employment on short notice are encouraged to apply immediately by facsimile to **Dr Bill Cham, Department of Medicine, The University of Queensland, Clinical Sciences Building, Royal Brisbane Hospital, Australia 4029 (fax (61 7) 365 5462; tel (61 7) 365 5155).**

Equal Opportunity in Employment is University Policy. (W8423)A

CAMBRIDGE LABORATORY
John Innes Centre, Norwich

A Biochemist is required to work on lipid and protein biochemistry in oilseeds in the Department of Brassica and Oilseeds Research. The successful applicant will direct a research programme in the area of oilseed biochemistry and physiology in collaboration with other members of the Department.

The appointment will be either to the Higher Scientific Officer or Senior Scientific Officer grade depending on qualifications and experience. Candidates should have a first or upper second class Honours Degree in Biology/Biochemistry together with at least two years relevant postgraduate research for the Higher Scientific Officer position and at least four years relevant experience for the Senior Scientific Officer position. Ideally you should have a relevant post graduate qualification.

Salary will be on the scale £11,586 to £16,176 pa for appointment as Higher Scientific Officer and £14,381 to £20,467 for the Senior Scientific Officer grade. Non-contributory superannuation. Equal Opportunities Employer.

Applications with full cv together with the names of two/three referees should be sent to the Personnel Officer, John Innes Centre, Colney Lane, Norwich NR4 7UH quoting reference PB/345. Closing date for applications is 24 May 1991.

The Cambridge Laboratory is associated with the AFRC Institute of Plant Science Research. (6096)A

UNIVERSITY OF MANCHESTER
SCHOOL OF BIOLOGICAL SCIENCES

DEPARTMENT OF CELL AND STRUCTURAL BIOLOGY

Applications are invited for four posts in the new Cancer Research Campaign funded group working on the molecular basis of cell adhesion in normal and neoplastic epithelial cells. The aim is to elucidate the role and function of intercellular adhesion mechanisms in malignant invasion and metastasis, and epithelial morphogenesis. The posts, which are available from 1st October, 1991, for four years (renewable annually), are as follows: **2 Postdoctoral Fellowships:** Candidates should have an interest in molecular biology and experience of mammalian expression systems would be an advantage. Candidates with a strong background of cell biology/protein biochemistry will also be considered. Basic salaries will be in the range £11,399 – £18,165 p.a. as appropriate to experience. In addition these posts receive a Cancer Research Campaign salary enhancement. **1 Graduate Research Assistant:** Candidates should have a degree in some aspect of Biological Sciences and an interest in molecular biology, cell biology and/or biochemistry. Practical experience in any of these areas would be an advantage but is not essential. A suitable candidate would be permitted to register for a Ph.D. Salary range £11,399 – £13,495 p.a. according to experience. **Research Technician:** Candidates should preferably have some experience of tissue culture and/or electron microscopy, though candidates with a more general laboratory background will also be considered. The level of appointment will be up to and including grade D (£9,706 – £11,589 p.a.) depending on qualifications and experience. Further particulars may be obtained from **Mrs J. Wilson, Department of Cell and Structural Biology, School of Biological Sciences, Stopford Building, Oxford Road, Manchester M13 9PT. Tel: 061 275 5241/2.** Applications including a curriculum vitae and the names and addresses of two referees should be sent to Mrs Wilson by 31st May, 1991. *The University is an Equal Opportunity Employer.*

(6105)A

Cancer Research Campaign

UNIVERSITE DE
LAUSANNE

Notre Faculté des sciences
au concours deux postes

PROFESSEUR ORDINA
(FULL PROFESSOR)

en physique expérimentale
matière condensée

Entrée en fonction: octobre
ou à convenir.

Participation à l'enseigneme
l'Institut de physique expérim
en physique générale et physiq
solide. Infrastructre de recher
disposition (cryogénie, produ
de hauts champs magnétiques
tallurgie, centre de micro-ana

Les candidatures (avec c. v. e
de publications) doivent pa
avant le 31 août 1991 à M.
Bünzli, Doyen de la Facult
Sciences, Collège propédeu
CH-1015 Lausanne. Rense
ments complémentaires: M
Wanders, Section de physiqu
1015 Lausanne, tél 010 4121
23 84. Soucieuse de promo
l'accès des femmes à la carrièr
démique, l'Université encoura
candidatures féminines. (W842

THE UNIVERSITY OF BIRMINGHAM

FACULTY OF MEDICINE AND DENTISTRY

DEPARTMENT OF RHEUMATOLOGY AND IMMUNOLOGY

RESEARCH ASSOCIATE

Required for a two year post studying functional interactions between B and T lymphocytes. The project is a continuation of studies of anti-idiotypic antibodies for rheumatoid factor which cross react with T cell receptors. The successful applicant will join an active collaborative research group investigating mechanisms of immunoregulation.

Informal enquiries to Dr Mike Salmon 021-414 6781 or Dr Roy Jefferis 021-414 4063. Maximum starting salary £11,399.

Application forms (2 copies) returnable by 15 May 1991 and further particulars available from the Director of Staffing Services, The University of Birmingham, Edgbaston, Birmingham B15 2TT. Tel 021-414 6483 (24 hours). Quote ref M2074. The University is an equal opportunities employer.

(6087)A

FACULTY POSITION

University of California, Irvine

The Department of Physiology and Biophysics invites applications for a faculty position at the Assistant Professor (tenure track) level commencing as early as September 1991. We are seeking candidates with the ability to define a significant research area in eukaryotic cell physiology, using modern techniques of cell biology, molecular biology, biochemistry, or biophysics. Examples of research interests would include cell signalling, differentiation and growth control, regulation of gene expression, intracellular processing and sorting of biomolecules, membrane formation and turnover, and the relationship of molecular structure to biological function. The position also involves teaching of medical and graduate students. We will begin considering applications starting June 1, 1991.

Send curriculum vitae, a brief research description, and the names of three referees to **Michael D. Cahalan, Chairman, Department of Physiology and Biophysics, University of California, Irvine, CA 92717.**

The University of California, Irvine, is an Affirmative Action/Equal Opportunity Employer. (NW6577)A

COLUMBIA UNIVERSITY

POSTDOCTORAL POSITION IN HUMAN MOLECULAR GENETICS

Applications are invited for a postdoctoral scientist to join our gro
working on the genetic and physical mapping of hum
chromosomes and the mapping and characterization of hum
disease genes. The post is available immediately. Applicant mu
have a strong background in human genetics or molecular biolo
and Ph.D. in a related area. Experience in gene mapping techniqu
(library construction, marker development, DNA sequencing, PFG
FISH, etc.) will be advantageous. Position is funded for a period o
and possibly 3 years. Salary and fringe benefits are excellent.
**Please address inquiries plus curriculum vitae to: Dr. Conr
Gilliam, Molecular Genetics Laboratory, Columbia Universi
722 West 168th St., Box 23, New York, N.Y. 10032.**
EO/AA employer.

(NW6614)A

Coláiste na hOllscoile Corcaigh
University College Cork

PROFESSORSHIP OF PHYSICS

The Physics Department is a key pivotal department in the Colleg
providing full undergraduate and postgraduate degree pro
grammes. The teaching and research activities of the Departmen
involve pure and applied, experimental and theoretical physics.

The College is now seeking to fill the Professorship of Physics. Ap
plications are invited from individuals with proven record o
achievements in research and teaching. The successful applican
will have the opportunity of leading the Department in major expan
sion and development.

The College will later this year also be seeking to fill a Senior Lec
tureship post in the field of Solid State Physics, with opportunitie
for collabration with the National Microelectronics Research Centr
and with the Department of Electrical Engineering and Microelec
tronics. The advertisement for this post will appear in due course

For informal discussion contact Professor M A Moran. Tel: [+353-21
276871 ext 2257.

Salary scale: IR£37,394-IR£43,245 pa.

**Application forms and further details of the post may be ob
tained from Mr M F Kelleher, Secretary, University College
Cork, Ireland. Tel: [+353-21] 276071 ext 2364.**

*Latest date for receipt of completed application forms is Friday, 14
June, 1991.*

University College Cork is an Equal Opportunity Employer. (6112)A

UNIVERSITY OF CAMBRIDGE

University Lecturer in Biochemistry

Eukaryotic Gene Expression

University Lecturer in Biochemistry to take up appointment on 1 October 1991 or as soon as possible thereafter. Candidates should hold a PhD degree or the equivalent and have an outstanding record in research and enthusiasm for teaching. Candidates are sought with postdoctoral experience in molecular biology, protein synthesis or gene expression, and strong preference will be given to candidates with expertise in the mechanism and control of gene expression in higher or lower eukaryotes. Salary scale: £14,169-£21,852.

Further information and application form from: The Secretary (Dr D Franks), Appointments Committee, 19 Trumpington Street, Cambridge CB2 1QA. Tel: 0223 334999 Fax: 0223 332355.

Closing date: 1 June 1991.

The University follows an equal opportunities policy.

(6088)A

Post-doctoral position

An up to 3 year post-doctoral position is now available at the Institut de Chimie des Substances Naturelles, Gif sur Yvette, to participate in an ongoing program on the methodology and the applications of multinuclear, multidimensional (2D, 3D and up . . .) Nuclear Magnetic Resonance to the determination of structure and dynamics of small proteins and oligonucleotides. Available spectrometers fields: 400 (2) and 600 MHz.

The successful candidate will have preferentially obtained recently a PhD in Organic Chemistry, Biochemistry or Biophysics, if possible with some experience in high field NMR and affinities for development of computational methods. The salary will be of 12,000 FF/month.

Candidates are requested to send a detailed CV and the names of two referees to Dr E Guittet, Lab RMN, ICSN-CNRS, 91190 Gif sur Yvette, France.

(W8425)A

Research Scientist (Avian Endocrinology)

IMPROVE PERFORMANCE

$47K-$52K package

Division of Animal Production
Sydney, Australia

We wish to appoint a Research Scientist who can undertake research to improve reproductive performance of broiler female lines by understanding the relationship between growth and reproduction, and to provide a scientific framework for optimising physiological, nutritional and genetic inputs to meat chicken production. The appointment will be made for a term of three years. You will join a multidisciplinary research team to develop collaborative research projects in this field.

You will need the ability to initiate and carry out original research, to work effectively in a team, and to work closely with Australian poultry breeding companies. You should have a Ph.D or equivalent qualifications in the field of reproductive or metabolic endocrinology.

Dr B.H. Yoo can provide more details of the job, our selection criteria, career opportunities and answer any questions you might have about this position. You can telephone Dr Yoo on (02) 886 4888 or contact him by Fax on (02) 888 9271.

Please send your application including details of your skills, qualifications and work achievements, marked "Confidential", to: **The Chief, CSIRO Division of Animal Production, PO Box 239, Blacktown NSW 2148.** Closing date for applications: 24th May 1991.

CSIRO
AUSTRALIA

CSIRO IS AN
EQUAL OPPORTUNITY EMPLOYER

(W8419)A

PROVOST

Duke University invites applications and nominations for the position of Provost. The Provost at Duke is the chief academic officer of the University and reports directly to the President. Applicants should have experience as a tenured faculty member in a major college or university, as well as some academic and budgetary responsibility for a major component of an educational institution.

Interested persons should write to: **Roger C. Barr, Chair, Provost Search Committee, Department of Biomedical Engineering, 136 Engineering Building, Duke University, Durham, NC 27706.** Include a complete curriculum vitae with the names of four references. Applications should be received before August 15, 1991.

Duke University

Duke University Is An Equal Opportunity/
Affirmative Action Employer (NW6650)A

IMMUNEX®

ASSOCIATE DIRECTOR, CLINICAL RESEARCH

Immunex Corporation is a Seattle pharmaceutical company focused on the research, development, manufacturing, and marketing of products to treat immune system disorders. We are seeking qualified candidates to join our Clinical Affairs Group as an Associate Director, Clinical Research.

The successful candidate will be responsible for managing the direction, planning, execution and interpretation of human clinical phase I-III trials for products under development. This individual will establish and approve scientific methods for design and implementation of clinical protocols and final reports. This position interacts with various internal and externals groups to insure adherence to protocols, coordination of report submissions to the FDA and assists in the preparation of INDs and PLAs.

Board qualified M.Ds with post graduate training in infectious disease or clinical immunology plus a strong commitment to clinical research are encouraged to apply. Experience should include a minimum of 7 years related experience in the pharmaceutical industry, clinical research or specialty practice. Candidates with 2 plus years of directly related clinical trial experience with human therapeutic products in a pharmaceutical or biotechnology company, and previous experience designing and/or monitoring human clinical trials are preferred. Good communication skills and the ability to work in a collaborative environment are essential.

Immunex Corporation offers a fast paced, stimulating environment, competitive salaries and an excellent benefits package. For consideration, please submit C.V., names of three references, and a list of publications to:

IMMUNEX CORPORATION, Attn: Human Resources Dept., 51 University Street, Seattle, WA 98101, Fax: (206) 587-0606.

Equal Opportunity Employer

(NW6661)A

SCIENTIST I/II—#1388

HAO-High Altitude Observatory
Exempt Range: 58, $3,193-$4,790/mo
60, $3,687-$5,530/mo

DUTIES INCLUDE: This position is for a physical scientist who will pursue independent and collaborative research as a member of the High Altitude Observatory (HAO) scientific staff. HAO is a division of the National Center for Atmospheric Research, and its research is focused primarly on studies of two coupled natural physical systems: (1) the Sun and heliosphere and (2) the terrestrial upper atmosphere, ionosphere, and magnetosphere. Research at HAO is also carried out on related astrophysical systems.

The incumbent will initially be expected to carry out theoretical (with an emphasis on computational), interpretive, and/or experimental research in one or more of the following areas: (1) the structure, dynamics, and evolution of solar and stellar interiors, with particular emphasis on solar rotation, convection, and the solar magnetic field; (2) the structure and dynamics of the solar atmosphere, with particular emphasis on the solar magnetic field, its interaction with turbulent convection, and its role in the origin of solar activity; (3) dynamics of the coupled system comprising the terrestrial magnetosphere, ionosphere, and upper atmosphere. It is expected that the incumbent's work will lead to an active program of individual and collaborative research in solar-terrestrial physics and related astrophysics, including regular publication in referred journals, presentations at national and international scientific meetings, and collaboration with other researchers at HAO and in the university community.

REQUIREMENTS INCLUDE: PhD or equivalent in a physical science; demonstrated experience in theoretical (with an emphasis on computational), interpretive, and/or experimental research concerning physical and/or astrophysical systems; a record of publications in referred journals showing originality and creativity, and giving evidence of the applicant's research experience. Additional requirements for consideration at the Scientist II level: Substantially greater research experience in the study of physical and/or astrophysical systems (with a significantly more substantial publication record) than required for Scientist I. Also desired, but not required: Knowledge of problems of current interest in solar and/or magnetospheric physics, experience with computational, interpretive, and/or experimental work in solar and/or magnetospheric research. Materials required for application: Curriculum vitae; list of all scientific publications; addresses for 3 scientists familiary with applicant's work, and statement from the applicant regarding the nature of his or her research interests. NOTE: Scientist I and II appointments are for terms of up to 3 and 4 years, respectively. Individuals may be appointed to the next higher level in accordance with the UCAR Scientific Appointments Policy.

Send resume to: Searl G. Brier, UCAR Human Resources Office, PO Box 3000, Boulder, CO 80307. Please refer to job #1388. NOTE: APPLICATIONS FOR THIS POSITION WILL BE ACCEPTED UNTIL 5:00 P.M. ON JUNE 30, 1991.

UCAR has a strong commitment to the principle of diversity in all areas. In that spirit, we are interested in receiving applications from a broad spectrum of people, including women, minorities, and disabled individuals.

University Corporation for Atmospheric Research

(NW6662)A

Leicester University

DEPARTMENT OF BOTANY

RESEARCH ASSOCIATE IN PLANT MOLECULAR BIOLOGY

Applications are invited for a plant molecular biologist to investigate using plant transformation techniques, the varied regulatory roles of the phytochrome photoreceptor gene family. The successful applicant will be part of an AFRC link group between Dr G C Whitelam, University of Leicester, and Dr N P Harberd, Cambridge Laboratory, John Innes Centre for Plant Science Research, Norwich. Previous experience in molecular biology and/or plant transformation would be an advantage. The appointment will be made at the postdoctoral level at an appropriate point on the RA 1A scale (£11,399-£18,165 pa under review).

For further details contact: **Dr Garry Whitelam, Department of Botany, University of Leicester, University Road, Leicester LE1 7RH (Tel: 0533 523396) or Dr Nic Harberd, Cambridge Laboratory, JICPSR, Norwich (tel: 0603 52571 ext 2525).** (6097)A

ANIMAL HEALTH TRUST

has a vacancy for a

VETERINARY BACTERIOLOGIST

We are seeking a microbiologist to head the Bacteriology Sectic within the Trust's Department of Infectious Diseases. Tl appointee will be expected to establish a research programm on equine bacterial diseases. The Department has an interest the pathogenesis, diagnosis, prevention and treatment respiratory disease, infectious causes of abortion and fo diarrhoea. The appointee will also be responsible for tl supervision of a comprehensive diagnostic laboratory. Tl Department is well equipped and has active researc programmes in molecular biology, virology, immunology ar pathology.

The laboratories are situated at Lanwades Park, Kennett, fi miles from Newmarket.

The appointment will be ont he University salary scale f Research and Analagous Staff. Starting salary will be up to maximum of £26,471 according to age and experience.

Informal enquiries may be made to
Dr J A Mumford, Head of Department (0638 750659)

Application forms available from
Personnel Officer, Animal Health Trust
PO Box 5, Newmarket, Suffolk CB8 7DW
(0638 661111 — quoting ref: 56/90)

(6100)A

The Hebrew University-Hadassah Medical School
Jerusalem

FACULTY POSITIONS

The Faculty of Medicine invites applications for Tenur Track Faculty Positions and for Allon Fellowship cand dates leading to Tenure Track Positions in Basic Medic Sciences.

Preference will be given to candidates that utilize moder molecular approaches in the following areas: Medic Microbiology, Human Genetics, Parasitology, Biochem istry, Cell Biology, Physiological Chemistry, Physiolog and Pharmaceutical Sciences.

PhD and/or MD degree and Post-Doctoral training ar required. Excellence in research and teaching will be prime importance in the decisions of the search commi tee. Candidates are expected to develop independer research.

Curriculum vitae, list of publications, 3-5 selecte reprints, research plans and three letters of recom mendation addressed to the Dean, Prof S Razir should reach the office of the Dean (POB 1172, 9101 Jerusalem) not later than September 30, 1991. (W8427)A

UNIVERSITY OF SOUTHAMPTON

Postdoctoral appointment in Superconductivity

Applications are invited for an SERC funded postdoctora research assistantship on a project to investigate the curren carrying properties of high T_c superconductors. The pos involves the study of the basic characteristics of the vortex stat and the pinning processes in a variety of materials (singl crystals, films, meltgrown materials) using magnetometry (12 VSM 5T SQUID magnetometer), magneto-transport and MuS studies.

Applicant should have a background in experimental soli state physics. The post is funded for two years with possibl extension. Initial salary in the range of £12,086-£14,74 according to age and experience.

Applications (2 copies) a curriculum vitae and the names an addresses of two referees should be sent to S/268, Personne Department, Southampton University, Southampton SO9 5N by 31st May 1991.

Working for Equal Opportunities. (6107)A

International Research Laboratories (IRL)
CIBA-CEIGY (Japan) Ltd.

IRL is one of the fundamental research organizations of CIBA-GEIGY Group, established in 1986 in Japan. The total number of research scientists is about 80 at present, and will be further increased to 130 toward 1994 in the laboratories of Bio-organics, New Materials, Analytics & Informatics and Chemistry. Now, IRL offers:

One Postdoctoral Position

in the field of

Signal Transduction Mechanism in Invertebrates

for a period of one to two years beginning in July/August 1991

Essential qualifications: — Ph.D. of 35 years old or younger — ability in English and/or Japanese communication.

We are looking for one postdoctoral scientist to undertake research into the molecular mechanisms controlling signal transduction in invertebrates. Experience in molecular neurobiology of electrophysiology would be an advantage.

Applications are invited by May 31st 1991 to the following address together with curriculum vitae, a publication list, concise description of interested research field and recommendation letters from two professional referees.

M. Shiono, Planning & Coordination, CIBA-GEIGY (Japan) Ltd, 10-66, Miyuki-cho, Takarazuka, Hyogo-Pref. JAPAN. Tel. 0797-74-2458 Fax. 0797-74-2325. (W8417)A

RESEARCH ASSOCIATE

PhD in organic chemistry

Classical carbohydrate manipulation and organic synthetic methodologies relating to complex molecules. Must be familiar with the use of analytical procedures, such as proton and carbon NMR, UV, LR, GC/MS, LC, TLC, computer-assisted molecular modeling for structure determination of complex natural products. Publication record showing independent research required. Salary 22,350, 40 hrs, if not a US citizen indicate visa status and basis for work authorization in the US.

Apply: Employment Security Commission, 516 North Mangum St, Durham, NC 27702 or your local Job Service Office. Refer to DOT Code 090-227-010. Job Order Number NC300/644 with copy of the advertisement within 30 days. (NW6660)A

---

nature
— the professionals' choice

UNIVERSITY OF MANCHESTER
SCHOOL OF BIOLOGICAL SCIENCES

Applications are invited for three lectureships in the School, available from 1st October 1991, as follows:

1. Lectureship in Developmental/Molecular/Cell Biology (Ref. No. 97/91.)

(Department of Cell & Structural Biology)
This post offers unrivalled opportunities to establish an excellent research programme in any area of developmental, cell or molecular biology. The Department is extremely well equipped and staffed, has a lively research atmosphere with numerous PhD students and postdoctoral research assistants and excellent local and international collaborations. Major research strengths include: cell matrix interactions, transcription factors, especially homeobox genes, cell adhesion, sex determination, normal and abnormal craniofacial development, soluble growth and migration factors, wound healing and neurobiology. Teaching duties will include courses in tissue/cell/developmental biology to dental students and embryology to medical students. We seek a dynamic, enthusiastic individual with any relevant scientific background.
Informal enquiries about the post may be made to Professor M. W. J. Ferguson (Tel: 061-275 6775).

2. Lectureship in Cancer Cell Molecular Biology (Ref. No. 98/91.)

(Department of Physiological Sciences)
Applications are requested from candidates with a proven ability to address questions in cancer cell biology by the use of techniques in molecular biology and genetic engineering. He or she should be able to generate independent, and preferably complimentary, programmes of work in the context of a group interested in molecular mechanisms of toxicant- and drug-induced programmed cell death and drug-induced cancer cell differentiation and to contribute to the teaching of science, medical and dental students. Excellent opportunities exist for collaborations within the Molecular Pharmacology and Toxicology group, with members of the School of Biological Sciences, the nearby Paterson Institute of Cancer Research, ICI Pharmaceuticals and ICI Central Toxicology Laboratories.
Informal enquiries about the post may be made to Professor J.A. Hickman (061-275 5617).

3. Lectureship in Physiological Sciences (Ref. No. 99/91.)

(Department of Physiological Sciences)
This post offers opportunities to develop independent research within one of the major research groups within the Department. Areas of strength include pharmacological and physiological aspects of neuroscience, chronobiology, epithelial transport and secretion, molecular pharmacology, renal function and smooth muscle actions. We are seeking a candidate of proven research ability, and it will be an advantage if he/she has some experience of teaching. While preference will be given to candidates working in neuroscience, renal function or smooth muscle action, this should not be regarded as exclusive. There is a large population of PhD students and post-doctoral fellows, and excellent opportunities for collaborative work with other areas in the School of Biological Sciences, with clinical departments and with industry and international groups.
Informal enquiries about the post may be made to Professor Roger Green (Tel: 061-275 5408).
Salary range £12,086 — £16,755 or £17,455 — £22,311 p.a. depending on experience. Particulars and application forms (returnable by May 20th) from the Registrar (Academic Staffing Office), the University, Manchester M13 9PL (Tel: 061-275 2028). Quote appropriate ref. no. *The University is an Equal Opportunity Employer.* (6106)A

Universiteit van Amsterdam

The Faculty of Biology has a vacancy for a

professor (m/f) in Plant Pathology N.8351.

full-time

The position is within the Department of Molecular Cell Biology.

Applications are invited for a tenured appointment to augment molecular-biological and cell-biological approaches in the study of plant-pathogen interactions.

Requirements and qualifications:

- extensive knowledge of plant pathology, especially of the molecular-biological and cell-biological aspects of plant pathogen interactions
- proven experience in molecular-biology, particularly in those aspects relating to plant pathology. Ability to initiate novel approaches in the study of plant pathogen interactions
- experience in the organization of and teaching in advanced undergraduate and graduate courses and supervision of PhD and MSc students
- experience in writing grant applications and in obtaining external financial support
- organizing ability.

The successful applicant will be expected to learn the Dutch language within two years.

The gross salary depends on experience and ranges from Dfl. 7.111,- to Dfl. 10.544,- per month (Dutch Civil Servants Code).

Further information can be obtained from the secretary of the nomination committee, Prof. Dr. H. van den Ende, Department of Molecular Cell Biology, University of Amsterdam, Kruislaan 318, 1098 SM Amsterdam, The Netherlands (telefax 031 - 20 525.7715) to whom applications with a curriculum vitae should also be sent - within 3 weeks. A statement of research interests and the names of two referees should be forwarded.

Women in particular are invited to apply.

(W8413)A

President & Scientific Director

The John P. Robarts Research Institute

The John P. Robarts Research Institute, an independent medical research institute affiliated with the University of Western Ontario and University Hospital in London, Ontario, Canada is seeking a new President and Scientific Director to take office in the summer of 1992. Reporting to the Board of Directors, the President and Scientific Director is the Chief Executive Officer and has both scientific and administrative responsibility (through a Vice-President, Administration) for the Institute.

Currently, there are three Research Groups related to specific diseases and the functioning of specific organs of the body (Heart and Circulation, Stroke and Aging, Immunology and Transplantation). There are also three Research Groups that are technology-oriented: Clinical Pharmacology, Advanced Imaging Research, and the Clinical Trials Resources Group.

Applications are requested from individuals who have a scientific background that is, or has been, recognized internationally in basic and/or clinical science. Individuals who have taken a leadership role in a scientific environment that encourages cooperation between basic scientists and clinicians will find the Institute a particularly rewarding environment. This is an outstanding opportunity to achieve personal growth and satisfaction in an internationally recognized research institute.

In accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents. Expressions of interest should be accompanied by a curriculum vitae, and will be treated in absolute professional confidence. Please direct your response to the address shown to the left.

The John P. Robarts
RESEARCH
INSTITUTE

**The
LANDMARK
Consulting
Group Inc.**

155 University Avenue
Suite 206
Toronto, Ontario M5H 3B7
Fax (416) 359-1882

(NW6648)A

HISTOLOGY LECTURERS

Widely approved, successful, internationa Medical School seeks qualified teaching staff Both senior and junior posts are available. Recently retired lecture will be considered. Abili to cross-teach in other areas is a plus.

Send C.V. (preferably b FAX) to

**Faculty Recruitment Office
St George's Universit
School of Medicine
GRENADA, West Indie
Fax 809 444-4823**

(NW6617)

POSTDOCTORAL POSITION

available immediately for up to 4 year study mechanism of biosynthesis, p cessing, and the regulation of releas cholecystokinin. Project uses molec biological, biochemical, and pharma logical approaches. Some experienc one of these areas is preferred. S *curriculum vitae and names of three erences to* **Dr M C Beinfeld, Dept. Pharmacol. & Physiol. Sci., Saint Lo U. Med. Ctr. 1402 S. Grand Blvd. Louis, MO 63104.** An affirmative acti equal opportunity employer. (NW664

Yamanouchi Research Institute

The Yamanouchi Pharmaceutical Co Ltd is one of Japan's most successful pharmaceutical companies, with an enviable reputation of new drug discovery stemming from its commitment to research. Part of its international expansion is a Life Science Laboratory – the Yamanouchi Research Institute.

The Institute, located in newly refurbished premises on the outskirts of Oxford, is conducting research on the control of cell behaviour in pathological processes, with a view to identifying targets for therapeutic intervention.
The appointments open are:-

Research Scientist *£15-25,000*

We are looking for a Research Scientist to work on projects on the interaction of leucocytes with endothelial cells and the extracellular matrix. Applicants should have up to 3 years post-doctoral experience and expertise in tissue culture and protein chemistry.

Research Technician *£11-13,000*

The technician will provide assistance to post-doctoral staff in tissue culture and protein chemistry. Applicants should have BTec or HTec or equivalent and 1-2 years laboratory experience, preferably relevant to this position. The opportunity for further training will be available.

Prospects for advancement are excellent and this is an ideal opportunity for enthusiastic and ambitious people to work with the very latest equipment in this exciting new Institute.
Benefits include free BUPA, free Life Assurance and a non-contributory pension.
Please send a full Curriculum Vitae to **Nigel Wild**, Yamanouchi Research Institute (U.K.), Littlemore Hospital, Oxford OX4 4XN. Tel: 0865 777228 Fax: 0865 778811.

(W8428)A

Yamanouchi
Research Institute (U.K.)

MICROBIOLOGIST/ HISTOPATHOLOGIST

The U.S. Fish and Wildlife Service, Longview, WA, seeks a microbiologist/histopathologist GS-11/12 to research the effects of nutrition, disease organisms, and fish cultural practices on the health and histology of anadromous fishes. Salary range $31,116 to $48,481.

Call Julie Swanberg at 503-231-6136 for job description, qualifications required and application forms. Deadline: 6/30/91. Equal opportunity employer. (NW6613)A

HARVARD POSTDOC

OPPORTUNITY IN GROWTH FACTOR RECEPTOR/PROTEIN KINASE C SIGNALLING

Expertise in recombinant DNA technology, protein chemistry, mammalian cell biology (1986 Nature 324, 68; 1987 Science 236, 197; 1988 PNAS 85, 1477; 1989 EMBO J. 8, 2943) or yeast biology desired.

Please send CV, summary of your background and interest and names of 3 referees or call: **Heimo Riedel, PhD, Assistant Professor of Medicine (Biochemistry), Joslin Diabetes Center, 1 Joslin Pl, Boston, MA02215; (617) 732-2691/2/3.**

(NW6652)A

COTTON-TOP TAMARINS PRIMATE DISEASE MODEL

The Center for the Study of Inflammatory Bowel Disease (CSIBD) at the MGH and the NERPRC maintains a large colony of Cotton-Top Tamarins (CTT) (*Saginus oedipus*) for use in research related to inflammatory bowel disease and colon cancer. Funded investigators interested in undertaking research projects involving non-terminal studies in the CTT are encouraged to contact the Primate Model Core Steering Committee, CSIBD, Massachusetts General Hospital, Jackson 7, Boston, MA 02114 or call (617) 726-7438. (NW6655)A

POSTDOCTORAL POSITION IN REPRODUCTIVE IMMUNOLOGY

To study immigration of lymphoid cells into the uterus during the ovarian cycle and pregnancy as well as the influences of lymphoid cell populations on placental growth and pregnancy success in rodent model systems. NIH-funded, with starting annual salary of $21,000. Available July 1, 1991. Please send curriculum vitae and names and addresses of three references to: **Dr. Judith Head, Cecil H. and Ida Green, Center for Reproductive Biology Sciences, The University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Boulevard, Dallas, TX 75235-0951. The University of Texas Southwestern Medical Center at Dallas is an equal opportunity/affirmative action employer.**

(NW6638)A

NSERC

Women's Faculty Award

The **Department of Geology of McMaster University** is looking for a candidate for an NSERC Women's Faculty Award for the Academic year 1992-93. The Department of Geology at present consists of eleven full-time, five part-time and two emeritus professors, plus six associate members in other departments. We have recognized strengths in the fields of geochemistry, both elemental and isotopic; clastic sedimentology; and biogeology. We have an ongoing commitment to expand our instruction and research in the field of environmental geology and geochemistry.

Emphasis would be put on evidence of proven record of accomplishment. Candidates should have completed a PhD degree or expect to have completed it by the time that they would take up the position in the summer of 1992.

Applicants should provide the names of two referees who can attest to their abilities in research and teaching.

Preference will be given to Canadian citizens or Landed Immigrants.

Interested applicants should write to Dr A P Dickin, Chair, WFA Search Committee, Department of Geology, McMaster University, Hamilton, Ontario L8S 4M1.

Deadline for receipt of applications is September 1st, 1991.

(NW6653)A

POSTDOCTORAL POSITION

MOLECULAR CELL BIOLOGY AND VIROLOGY

We have a postdoctoral position immediately available at the Novum Research Center, Karolinska Institute (Stockholm). The project will involve work on any of the following topics: (i) assembly and entry mechanism of animal membrane viruses, (ii) intracellular routing of proteins in animals cells, (iii) development of improved viral DNA expression vectors to be used for research, production and gene therapy.

Applications including CV, summary of earlier research activities together with the names and addresses of two referees should be sent to Professor Henrik Garoff, Department of Molecular Biology, Novum, S-141 57 Huddinge, Sweden.

(W8422)A

MEDICAL RESEARCH COUNCIL

NATIONAL INSTITUTE FOR MEDICAL RESEARCH

POSTDOCTORAL SCIENTISTS

Applications are sought for two postdoctoral positions of 2 and 3 years' duration respectively to conduct research on T-cell development. The research will focus on T-cell specific gene regulation as well as cellular interactions at the molecular level in the developing thymus. Applicants with experience in techniques of recombinant DNA and tissue culture are encouraged.

The two year position (Ref: GSE/PD/EC) is available immediatley and will be funded by the European Community; applicants for this position *must* be citizens of EC member states. The three year position (Ref: GSE/0572) will be funded by the MRC and will be available from August 1991. The starting salary for both posts will be in the range £15,085 to £24,078 — pay award pending — inclusive of London Allowance (dependent on experience). MRC Pension Scheme option.

Applications including a curriculum vitae, list of publications and two professional referees should be sent to Mr C R Russell, Administrative Manager, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, not later than 28th June 1991 quoting the relevant reference number. Further details and informal enquiries about the position are welcome and may be made to Dr Kioussis on 081-959 3666 ext 2113.

The MRC is an Equal Opportunities Employer

Smoking is actively discouraged.

MRC

(6094)A

TWO POSITIONS — RESEARCH ASSOCIATE

in peptide design and synthesis: 1) bradykinin antagonists 2) helizyme enzymes. Require chemistry PhD, experience solid phase synthesis. Send resume and references by May 10, 1991 to:

Dr John Stewart,
Biochemistry, B-121,
UCHSC, 4200 E 9th Ave, Denver,
CO 80262.

AA/EOE employer.

(NW6647)A

THE UNIVERSITY OF SHEFFIELD

Department of Physics

LECTURER IN ASTRONOMY

Applications are invited for the above post which is available from the 1st October 1991.

The University of Sheffield has very popular dual honours degrees in Astronomy / Physics, Astronomy/Mathematics and Astronomy/Chemistry. The appointee will be expected to teach undergraduate courses in astronomy and help run the undergraduate practical observing sessions.

Candidates whose research field is observational astronomy are being sought and preference will be given to someone interested in the broad areas of Stellar Evolution, Galactic Structure or Cosmology. In addition to carrying out their own work the successful applicant will be encouraged to supervise post-graduate students and to build up an active group which will provide a strong new thrust to astronomy research within the department.

Salary on the scale for lecturers (£12,086-£22,311 pa) depending upon age and experience.

Further details from Director of Personnel Services, The University, PO Box 594, Firth Court, Western Bank, Sheffield S10 2UH (Tel: 0742 768555 ext 4144) to whom applications including a full CV and the names/addresses of three referees (six copies of all documents) should be sent by 17 May 1991. **Informal enquiries to Dr David W Hughes or Dr Fred Combley at the Physics Department (Tel: 0742 768555). Please quote ref: MAP386/G.**

An Equal Opportunity Employer.

(6080)A

UNIVERSITY OF DUBLIN

TRINITY COLLEG

LECTURER IN GENETICS

Applications are invited for above post in the Departmer Genetics at Trinity College, Du The Department has strong teac and research programmes in a b range of genetics. Research inte include: gene expression in *B su* gene expression in plants and viruses; molecular genetic ana of retinitis pigmentosa; the mo lar biology of inflammation; mo lar evolution and population g tics; animal genetics. Candidate asked to indicate the area of gen in which they would like to teach ideas for the future developme their research.

Salary Scale: IR£11,683-IR£26 per annum.

Appointment will be made withi salary range IR£12,683-IR£17 per annum, at a point to accord qualifications and experience date.

Application forms and further ticulars relating to this post ma obtained from: **Establishment ficer, Staff Office, Trinity Coll Dublin 2. Tel: 772941 ext 1678/ 772169** to whom completed app tion should be returned by not than Friday, 17th May, 1991.

Candidates may, if they wish, cor Professor David McConnell 772941 ext 2008/Fax 6798558) additional information.

TRINITY COLLEGE IS AN EQU OPPORTUNITIES EMPLOYE

(6104

UNIVERSITY OF DURHAM

SCHOOL OF ENGINEERING AND APPLIED SCIENCE (APPLIED PHYSICS GROUP)

POSTDOCTORAL RESEARCH ASSISTANT

The post is in the area of Molecular Electronics and involves the characterisation of the interface between biological molecules and conducting polymers. This will be explored in a joint project with Tokyo Institute of Technology. The properties of films of oligo- and poly-pyrroles incorporating a variety of enzymes will be studied.

Applicants with backgrounds in Biological Sciences, Physical Chemistry or Materials Science would be welcomed.

The post is tenable from as soon as possible until 31 October 1993, with a period of about six months in Tokyo. Salary in the range £11,399-£13,495 pa (under review) on the 1A salary scale for Research and Analogous Staff.

Please direct informal enquiries to Professor D Bloor (tel. 091 374 2391).

Further details may be obtained from the Personnel Officer, Old Shire Hall, Durham DH1 3HP, tel. 091-374 4687, to whom applications should be sent not later than 17 May 1991. Please quote reference A002.

(6092)A

IVERSITY OF THE WEST INDIES

ave Hill Campus, Barbados

plications are invited for the
owing posts in the Centre for
ource Management and Envi-
mental Studies (CERMES):

FISHERIES AND RESOURCE MANAGEMENT

ESOURCE ECONOMIST

successful applicants will be
ected to assume duties as soon as
sible. The posts are at the level of
ior Lecturer/Lecturer. De-
ding on the qualifications and
erience of the applicants one ap-
ntment will be made at the level
Senior Lecturer and the other at
level of Lecturer. Proven re-
rch ability and capacity to attain
nt funding are essential, and the
lity to teach data management, a
owledge of microcomputer sys-
ns and Caribbean or other tropi-
experience are highly desirable.
ditional duties will include
ervision of students' projects
organising and conducting
ining workshops in the Carib-
an region. Salary Scales: Senior
cturer BDS$54,840 × 1776 –
,496 x 1920 – 71,256 (Bar) ×
20 – 75,096 p.a. Lecturer
S$41,316 × 1776 – 51,972
ar) × 1776 – 62,628 per annum.
to five full economy class pas-
ges plus baggage allowance of
$1,200 on appointment and nor-
l termination. Special allowance
to US$400 for shipment of aca-
mic books and teaching/research
uipment on appointment. Unfur-
hed accommodation at 10% of
sic salary or optional housing
owance of 20% of basic salary to
ff making own housing arrange-
nts. UWI contribution of equi-
lent of 10% of basic salary to
perannuation Scheme. Annual
udy and Travel Grant for self,
ouse and up to three children.
ok Grant up to BDS$850 per
num. Detailed applications
ree copies) giving full particulars
qualifications and experience,
te of birth, marital status and the
mes and addresses of three re-
rees should be sent as soon as
ssible to the Campus Registrar,
niversity of the West Indies, PO
x 64, Bridgetown, Barbados. The
niversity will send further particu-
rs for this post to all applicants.
ese particulars may also be ob-
ined from the Appointments Of-
er, Association of Common-
alth Universities, 36 Gordon
uare, London WC1H 0PF, UK.

(W8418)A

UNIVERSITY OF LONDON SCHOOL OF PARMACY

LECTURESHIP IN PHARMACEUTICAL CHEMISTRY

Applications are invited from pharmacists and pharmaceutically-trained chemists or biochemists for a Lectureship in the School with research interests in synthetic medicinal chemistry, pharmaceutical analysis, drug structure metabolism, macromolecular structure, drug macromolecular interactions or drug delivery and formulation. Candidates trained in other relevant areas are also encouraged to apply. Preference will be given to pharmacists.

The successful candidate will be required to teach chemical or biochemical sciences on the BPharm and the BSc Toxicology and Pharmacology undergraduate courses.

Salary on scale £12,086-£16,755 (Lecturer A) or £17,455-£22,311 (Lecturer B) plus £1,767 London Allowance. For an informal discussion about the post please contact Professor W A Gibbons (tel 071 753 5884). For further information about the School please contact Mr N J Rampley, Assistant Secretary (071 753 5814), the School of Pharmacy, 29-39 Brunswick Square, London WC1N 1AX to whom applications in the form of a curriculum vitae to include a proposed programem of research, and naming three referees should be submitted by no later than 31 May 1991.

(6069)A

PERKIN ELMER CETUS

PCR TECHNICAL SPECIALIST

Perkin Elmer Cetus, the leader in PCR technology, is committed to providing the most comprehensive PCR technical support possible worldwide. We are seeking an experienced molecular biologist for the position of PCR Technical Specialist in our European support center located in Munich. The ideal candidate, as part of the molecular biology research community, will interact with our customers as a scientific peer.

Daily responsibilities center on the support and education of users via seminars, workshops, production of technical literature, and telephone applications assistance. The primary responsibility is to ensure the optimal use of PCR instrument/reagent systems in a variety of application areas and maintain a dialog with the research community regarding product/technique improvements and new product ideas.

An M.S. or Ph.D. in molecular biology or directly related area is required. Recent laboratory experience in techniques for DNA isolation, manipulation, amplification and detection are essential. Effective communication and public speaking skills are required in order to educate researchers and sales personnel about PCR methodology, applications and related technologies. English and German fluency required; additional language skills a benefit. Extensive travel involved.

Interested candidates please send letter and complete C.V. with references to: Ann Sawyer, Perkin Elmer Bahnhofstrasse 30, D-8011 Vatersetten, Germany.
Closing date for submission of applications is 17 May, 1991.

(W8426)A

PERKIN ELMER CETUS

TRANSPLANTATION IMMUNOLOGIST

LOYOLA UNIVERSITY OF CHICAGO

The Loyola University of Chicago cardiac transplantation program has an immediate opening for an immunologist to establish a high quality basic research program in transplantation immunology. A tenure track will be granted in the Departments of Medicine and Microbiology and Immunology at the assistant or associate professor levels. Candidates should have a PhD and a record of established research in the areas of allograft rejection, transplantation tolerance and/or allograft vascular disease. Candidates must be willing to participate in graduate and medical students teaching. While the successful candidate is expected to obtain extramural funding, the salary is not dependent from grant funds.

Please submit curriculum vitae, 2 copies of relevant papers, description of research goals and three supporting letters to:

Maria Rosa Costanzo-Nordin, MD
Medical Director
Cardiac Transplant Program
Loyola University of Chicago
Maywood, Illinois 60153 USA

(NW6576)A

New Mexico State University has a collaborative research project on SIV in African green monkeys with the Institute of Primate Research (IPR), National Museums of Kenya, Nairobi, Kenya. Position is for a **PhD in Virology or Viral Epidemiology.** Opportunity exists for additional SIV related research. Housing and travel provided. Successful applicant will be employed by the Institute of Primate Research. For consideration, send CV to **Dr Preston A Marx, Director, NMSU Regional, Primate Research Laboratory, Holloman AFB, NM 88330-1027, Phone (505) 479-6101.**

(NW6646)A

UNIVERSITY OF CAMBRIDGE

Department of Zoology

POST-DOCTORAL RESEARCH ASSOCIATE

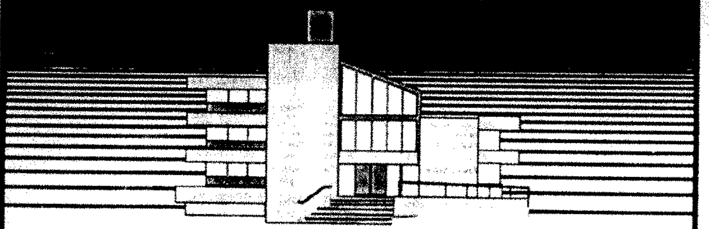
The associate will be involved with Professor G Horn and Professor P Bateson in a project on neural mechanisms of imprinting in chicks, supported by the AFRC. The post must be filled on or before 1st October 1991. The grant terminates 30th September 1993.

Salary, according to age, £11,399 to £16,755.

Apply not later than 24 May 1991 with a CV and the names of two referees to J Andrews, Department of Zoology, Downing Street, Cambridge CB2 3EJ. For further information call Prof P Bateson on (0954) 210301.

(6072)A

THE BIOMEDICAL RESEARCH CENTRE
Vancouver, British Columbia, Canada



POSTDOCTORAL POSITIONS

Applications are invited for two posts funded by The National Cancer Institute and The MRC of Canada for three years in the laboratory of Dr Steven Pelech. Applicants should have a strong, demonstrated interest in the study of cellular signal transduction by protein phosphorylation. The research focuses on the isolation and characterization of protein kinase cascades implicated in the control of cell cycle progression in oocytes or platelet activation. The projects involve protein kinase purification by automated column chromatography, and their enzymological characterization, microprotein and cDNA sequencing analysis, substrate and phosphorylation site determination, immunological characterization with novel antipeptide antibodies, and reconstitution by microinjection into oocytes. The multidisciplinary and highly interactive environment of The Biomedical Research Centre offers excellent potential for career development.

Applicants should have or will soon be completing a PhD. In accordance with Canadian Immigration requirements, preference will be given to Canadian citizens and landed immigrants, although all qualified individuals are encouraged to apply. They should submit a curriculum vitae, including the names, phone numbers and addresses of three referees, and a statement of their research interests to:

Dr Steven Pelech,
The Biomedical Research Centre,
2222 Health Sciences Mall,
Vancouver, BC, Canada V6T 1Z3.

(NW6654)A

SCHOOL OF PURE AND APPLIED BIOLOGY

LECTURER IN PLANT CELL BIOLOGY

(Fixed Term one year from 1 October 1991)

You will be expected to contribute lectures and practicals in the field of plant development and plant growth regulators at second year undergraduate level. The department has excellent cell biology facilities and you will have ample opportunity to engage in research.

You should have a PhD or have submitted your thesis in a relevant research area, prior to the start of the appointment.

Salary £12086 - £16755 per annum

For details please write to Personnel Division, 50 Park Place, Cardiff CF1 3AT or ring (0222) 874897. Please quote Ref 91/47.

Closing Date: 16/5/91.

CARDIFF
UNIVERSITY OF WALES

(6098)A

RESEARCH ASSISTANT PROFESSOR

Research on biochemistry and molecular genetics of *Vibrio* and *Acinetobacter*, and teaching bacterial genetics in graduate courses. **Requires:** PhD Biochemistry; 2 yrs postdoctoral experience in characterization of bacterial siderophores and membrane proteins; at least 10 yrs experience in biochemistry and microbiology research with emphasis on transcription and translation analysis in prokaryotic and eukaryotic systems; skills in DNA cloning and sequencing, molecular analysis of RNA, bacterial siderophores, and proteins; at least 10 yrs teaching biochemistry and bacterial genetics undergrad and grad students. Demonstration of success as independent scientist by (a) grant support in own name from peer-reviewed organizations, and (b) publishing 15 research papers at least 5 as primary author in peer-reviewed journals. Applications will be considered for a month after publication of this advertisement.

Application letter, CV, and names of 3 references to: Ms Judy Hoff, Dept of Microbiology & Immunology, L220, Oregon Health Sciences University, 3181 SW Sam Jackson Park Rd, Portland, OR 97201. *AA/EO Employer.* (NW6645)A

TECHNICIAN/ TECHNICAL ASSOCIATE

to conduct studies in the molecular biology of aging brain and Alzheimer's disease. Candidate should be experienced in quantitative studies of message levels in human and animal brain using RNA extraction methods. Northern and slot blots, solution hybridization and PCR. Also library screening, cloning, sequencing, *in situ* hybridization and subtractive hybridization. Some experience with western blotting and ELISA desirable. Willingness to develop and use a wide variety of techniques essential. Able to operate with minimal supervision once experimental design has been established in consultation with laboratory director. Contact: **Dr Paul Coleman, Dept of Neurobiology & Anatomy, University of Rochester, 601 Elmwood Ave, Rochester, NY 14642.** (NW6657)A

Research in Experimental Pathology and Molecular Pathobiology Leading to the PhD

Expanded faculty and state-of-the-art facilities; stipends of $13,000 and up per year including full tuition waivers; attractive campus in suburban Westchester close to New York City; expedited review process and flexible entrance times for promising candidates.

Contact: Dr Michael B Stemerman, Professor and Chairman, Department of Experimental Pathology, New York Medical College, Valhalla, NY 10595. Tel: 914-993 4146. EOE.

(NW6658)A

TECHNICIAN/ TECHNICAL ASSOCIATE

to conduct studies in the peptide biochemistry of Alzheimer's disease. Candidate should be experienced in preparation of tissue samples for HPLC analysis and possess good mechanical aptitude. Familiarity with western blotting and ELISA desirable. Willingness to develop and use a wide variety of techniques essential. Basic knowledge of IBM type computers and DOS important. Able to operate with minimal supervision once experimental design has been established in consultation with laboratory director. **Contact: Dr Paul Coleman, Dept of Neurobiology & Anatomy, University of Rochester, 601 Elmwood Ave, Rochester, NY 14642.** (NW6659)A

THE AUSTRALIAN
NATIONAL UNIVERSI

RESEARCH SCHOOL
BIOLOGICAL SCIENC

MOLECULAR
NEUROBIOLOGY GRO

POSTDOCTORAL FELL
RESEARCH FELLOW

A research position is availab
study embryonic developme
the inset neuromuscular sy
The work initially will be on g
hopper with possible extensi
Drosophila. Either a molecula
proach, examining the structur
distribution of homeobox gen
the developing neuromuscula
tem, or a cellular approach, i
ving ablation or blocking of put
guidance cues followed by em
culture, will be pursued depen
on the background of the succe
applicant. Position available
mediately. An appropriate P
required, with three years pos
toral research experience bein
sirable for appointment at the
search Fellow level. Enquiries
Eldon Ball, Molecular Neuro
ogy Group, RSBS, PO Box
Canberra, ACT 2601, Aus
(tel. (61 6) 249 4496; Fax (6
248 9995 or (61 6) 249 3784)

Closing date: 31 May 1991.
BS 18.4.1.

SALARY: Research F
A$33,163 – A$43,096 pa;
doctoral Fellow Grade 1 (
point) A$28,792-A$32,762
APPOINTMENT: Research
low up to three years, possibili
extension to five years; Postd
ral Fellow up to two years, p
bility of extension to three y
Enquiries welcomed from per
interested in secondment from
home institutions. APPLI
TIONS should be submitted in
plicate to the Registrar, The Au
lian National University, GPO
4, Canberra ACT 2601, Austr
quoting reference number an
cluding curriculum vitae, list of
lications and names of at least
referees. The University rese
the right not to make an app
ment or to appoint by invitatio
any time. Further informatio
available from the Registrar
from Appointments (39331), A
ciation of Commonwealth Uni
sities, 36 Gordon Square, Lon
WC1H 0PF.

THE UNIVERSITY IS
EQUAL OPPORTUNITY
PLOYER (W8420

---

nature
the widest
international
selection of jobs in
science —
EVERY WEEK

HARPER ADAMS

HEAD OF THE CROP PRODUCTION & SCIENCE DEPARTMENT

Applications are invited for this new and challenging appointment available from 1st September, 1991. The person appointed will be part of the College's Senior Management team and be responsible for the Department's teaching and research in support of post-graduate, degree and higher diploma level students.

The post offers immense scope for a person with the relevant higher degree and managerial flair supported by proven ability in the area of agronomy in teaching, research or industry.

The salary will be £29,777 and is reviewed annually by the Board of Governors.

Further particulars and application forms are available from: The Principal's Secretary, Harper Adams Agricutlural College, Edgmond, Newport, Shrophsire TF10 8NB. Tel: Newport (0952) 820280.

Closing date for applicaitons: 8th May, 1991 (6111)A

UNIVERSITY OF CAMBRIDGE

Department of Anatomy

RESEARCH TECHNICIAN

required now for project investigating neuronal control of movement. Tenure until 30th November 1993 in first instance. Skills required: sound histological background including immunohistochemical techniques. Salary £9,939-£12,227 according to age/experience.

Applications with CV and names of two referees to The Secretary, University of Cambridge Department of Anatomy, Downing Street, Cambridge CB2 3DY, by 10th May 1991. Telephone enquiries welcome (0223 333765). (6099)A

For

ADVICE

on placing a

Classified Advertisement

in Nature contact your

nearest Sales Office

Appointments · Conferences · Awards · Fellowships · Grants · Workshops

London	Tel: 071-872 0102	Fax: 071-240 2408
Paris	Tel: (1) 48 87 24 43	Fax: (1) 48 87 46 71
Munich	Tel: (089) 52 40 82	Fax: (089) 5 23 22 22
Tokyo	Tel: (03) 3267 8751	Fax: (03) 3267 8746
New York	Tel: (212) 477 9625	Fax: (212) 505 1364
Toronto	Tel: (416) 690 2423	Fax: (416) 868 1213
San Francisco	Tel: (415) 781 3803	Fax: (415) 781 3805

Booking is Easy Advice is Free

nature

INTERNATIONAL WEEKLY JOURNAL OF SCIENCE

UNIVERSITY OF OXFORD
SIR WILLIAM DUNN SCHOOL OF PATHOLOGY
RESEARCH ASSISTANT

Academic-related research staff grade 1B; Salary £10,700-£15,444

A research assistant is required to work with Dr Jasper Rees on the role of the cytoskeletal protein talin in the action of cytotoxic and helper T cells. The project will concentrate on the use of homologous recombination to disrupt the normal talin gene, followed by the analysis of talin function using transfection of cDNA clones. Further characterization by protein biochemistry will also be necessary. Applicants must possess an appropriate science degree and have experience of cell culture techniques, molecular biology or protein biochemistry. The position is funded by a grant from the Wellcome Trust until 31 October 1993 with the possibility of extension for a further two years. **Applications in writing, together with cv and names and addresses of two referees, should be sent to The Administrator, Sir William Dunn School of Pathology, South Parks Road, Oxford OX1 3RE. Closing date for applications: 17th May, 1991. The University is an equal opportunity employer.** (6109)A

---

nature

— the professionals' choice

FELLOWSHIPS/ STUDENTSHIPS

UNIVERSITY OF DUNDEE

Department of Biochemistry

TEACHING FELLOWSHIP

Persons with interest and experience in the teaching of Biochemistry are invited to apply for the above position which is available for a period of 2 years. The position will involve assisting in the development and presentation of laboratory and demonstration classes in the introductory science and first year pre-clinical Biochemistry classes. The Appointee will be a full member of the teaching teams for these courses. There is considerable scope for the development and production of video recorded self-learning packages. There will be an opportunity for some lecturing. The Department has an international reputation for the production of innovative teaching material. Persons wishing to work on a part-time basis will be considered.

Further particulars from, and application in writing with CV (6 copies or, if posted overseas, one copy in a format suitable for photocopying) and the names and addresses of three referees to the **Personnel Office, The University, Dundee, DD1 4HN. Please quote reference EST/6/91/N.** Closing date: Thursday 23rd May, 1991.

UNIVERSITY OF READING

School of Plant Sciences

Applications are invited for an AFRC-funded Postgraduate Research Studentship on the role of intracellular calcium and protein phosphorylation in the regulation of cytoskeletal function in plant cells. The successful applicant will join an active research group investigating calcium control mechanisms in plants. Applicants should have or expect to obtain a first or upper second class honours degree in a biological or biochemical science subject. The average stipend for the appointed student will be £5,000 pa from October 1991.

Applicants should send a full curriculum vitae and names and addresses of at least two referees to: **Dr Nick Battey, Plant Science Laboratories, University of Reading, Whiteknights, Reading RG6 2AS (Tel: 0734 318071; Fax: 0734 750630) as soon as possible. Further details of the studentship can be obtained from this address.** (6084)F

THE UNIVERSITY OF BIRMINGHAM

School of Medical Science

Department of Immunology

MRC-FUNDED RESEARCH STUDENTSHIP

Tenable from 1 October 1991. The project is to study mechanisms of suppression of programmed cell death of human B cells. The successful candidate will join an active multidisciplinary research department which includes groups invovled in studies of B cell signalling and differentiation, antibody structure and experimental haemopoiesis. Candidates should possess, or expect to obtain, a First or Upper Second Class Honours Degree in a biological science.

For further information, contact Dr Chris Gregory on 021 414 4066/4033.

Additional written details and application forms (returnable by 31 May 1991) available from: The Senior Assistant Registrar (HD), Medicine and Dentistry Divison, The University of Birmingham, Edgbaston, Birmingham B15 2TT, UK. (Tel: 021 414 4051).

The University is an equal opportunities employer. (6076)F

BRITISH DIABETIC ASSOCIATION RESEARCH STUDENTSHIPS

Applications are invited for two studentships recently awarded by this Association. The studentships will be of three years duration and should lead to the successful candidate achieving a PhD. Both studentships are available from 1st October 1991 and applications should be made to the respective Universities as detailed below, enclosing a curriculum vitae and the names of two academic referees.

1. **UNIVERSITY OF BIRMINGHAM**
   "Structural charactisation of the molecular interfaces and response involved in the interaction of insulin with its receptor"

   This project will investigate the structural basis for the interaction of insulin with its receptor and will involve training in techniques of protein chemistry and molecular biology and entail the use of high resolution nuclear magnetic resonance spectroscopy and molecular graphics to analyse structure-function correlations.

   Candidates should have (or expect to obtain) a good honours degree in Biochemistry or a related subject. The annual maintenance grant will be £6,600. Applications should be sent to **Dr B A Levine, School of Biochemistry, University of Birmingham, Birmingham B15 2TT, Telephone 021 414 5402** from whom further details may be obtained. The studentship offers an excellent opportunity to work closely with research teams examining aspects of signal transduction at the School of Biochemistry and the Medical School.

2. **UNIVERSITY OF LONDON, ST. GEORGE'S HOSPITAL MEDICAL SCHOOL**
   "Adrenergic mechanisms affecting glucose and amino acid uptake"

   A postgraduate physiologist/pharmacologist with a 1st or 2nd class honours degree is required to undertake a project concerned with in vivo and in vitro studies of adrenergic mechanisms affecting glucose and amino acid uptake. The annual maintenance grant will be £7,300. Potential applicants can obtain further information by contacting **Professor M J Stock at the Department of Physiology, St. George's Hospital Medical School, Tooting, London SW17 0RE, Telephone 081 672 9944 Ext 55357.**

A charity helping people with diabetes and supporting diabetes research. (6077)F

The Laboratoire d'Immunobiologie Moléculaire of the Ecole Normale Supérieure de Lyon plans to offer a:

POST-DOCTORAL FELLOWSHIP

Applicant with good Molecular Biology background is invited to join a research programme on Major Histocompatibility Complex class II and Invariant chain gene expression and antigen presentation. The position will be tenable before December 31st. Applicants should send a copy of their C.V., including the names and addresses of three referees to Chantal Rabourdin-Combe, Immunobiologie Moléculaire, CNRS-ENS UMR49, 46 Allée d'Italie, 69364 Lyon cedex 07, France, (Fax: 33 72 72 80 80). Applications should not arrive later than 25th May 1991. (W8415)E

POSTDOCTORAL POSITION IN POLAR BIOLOGICAL OCEANOGRAPHY

The Hancock Institute for Marine Studies (HIMS) offers a one-year (renewable for second year) postdoctoral fellowship to pursue original research from July 1991. Research is focused on microbial/phytoplankton interactions and carbon cycling and candidates will pursue their field studies in Arctic or Antarctic seas.

Send letters of interest and reference and curriculum vitae to Professor Cornelius W Sullivan, Graduate Program in Ocean Science, University of Southern California, University Park, Los Angeles, CA 90089-0373.

An affirmative action/equal opportunity employer. (NW6639)E

UNIVERSITY OF BRISTOL
DEPARTMENT OF VETERINARY MEDICINE
(MUSCLE & COLLAGEN RESEARCH GROUP)
AFRC PhD STUDENTSHIP
A Study of the Collagenous Matrix of Avian Bone

Funded by the Agricultural & Food Research Council and tenable for three years with a stipend of £5,000 in the first year and annual increments thereafter.

The project will study the biochemistry of bone collagen, investigating changes in bone structure, composition, degradative enzymes, and the relationship between osteoclasts and osteoblasts that leads to bone fragility in hens.

Applications are invited from candidates who hold or expect to hold a First or Upper Second Class Honours degree in a relevant subject. Full CV and the names of two academic referees should be sent to Professor AJ Bailey, Muscle & Collagen Research Group, Department of Veterinary Medicine, University of Bristol, Langford, Bristol BS18 7DY. (6079)F

POSTDOCTORAL FELLOWSHIP

Available July 1991 to study the molecular and cellular biology of insulin action. Specific projects include structure/function relationship for the insulin receptor, the cellular trafficking of glucose transporter (GLUT 4) enriched vesicles, and the regulation and expression of genes conferring insulin responsiveness on the target cells of muscle and fat.

Please send a curriculum vitae and the names of 3 references to: **Dr Paul F Pilch, Department of Biochemistry, Boston University School of Medicine, 80 E Concord St, Boston, MA 02118.** (NW6649)E

THE AUSTRALIAN
NATIONAL UNIVERSI
RESEARCH SCHOOL (
PHYSICAL SCIENCES A
ENGINEERING
POSTDOCTORAL FELLOWSHIPS

These fellowships are intende
those who have recently compl
or expect by 31 December 19
complete, requirements for a
degree or equivalent qualifica
Successful appointees will be
pected to take up their positio
the middle of 1992. Appointm
will be for two years only and
be in any field of research of int
to the School. The School co
of a General Physics Div
(applied mathematics, atomic
molecular physics, laser phy
optical sciences, plasma rese
electronic materials enginee
systems engineering), and de
ments of Nuclear Physics
Theoretical Physics.

Closing date: 30 June 1991.
PSE 9.4.1.

SALARY: Postdoctoral Fe
Grade 1 (fixed point); A$28,7
A$32,762 p.a. Postdoctoral Fe
Grade 2; A$33,163 — A$43
p.a.; APPLICATIONS shoul
submitted in duplicate to the R
trar, The Australian Nati
University, GPO Box 4, Canb
ACT 2601, Australia, quo
reference number and inclu
curriculum vitae, list of publicat
and names of at least three refe
The University reserves the
not to make an appointment
appoint by invitation at any t
Further information is avail
from the Registrar; or f
Appointments (39298), Ass
ation of Commonwealth Uni
sities, 36 Gordon Square, Lon
WC1H 0PF.

The University is an Equal Op
tunity Employer. (W841

continued on p

PRIZES

BRITISH DIABETIC ASSOCIATION
and
St BARTHOLOMEW'S HOSPITAL
ANDREW CUDWORTH MEMORIAL PRIZE

To commenorate the work of the late Professor Andrew Cudworth, the British Diabetic Association, in association with the Department of Medicine, St. Bartholomew's Hospital, is offering an annual prize of £500 for the best assay submitted on any aspect of diabetes. The prize winner will be invited to present the winning paper at a BDA Medical and Scientific Section Conference, and his/her travel, accommodation and registration fees for the Conference will be met by the Association.

Applicants should be under 40 years of age. Essays should be no longer than 10,000 words, and 4 copies should be sent to: Nick Grundy, Secretariat, British Diabetic Association, 10 Queen Anne Street, London W1M 0BD, before 5th July 1991.

"A charity helping people with diabetes and supporting diabetes research" (6071)U

continued on p

UNIVERSITY OF EDINBURGH

Department of Physiology

Post-Doctoral Research Fellowship

Developmental Neurobiology

post-doctoral research fellow-
hip is available for two years from
nuary 1992 in a project funded
y the Wellcome Trust on the
evelopment, maintenance and
egeneration of neuromuscular
ynaptic connections. The re-
earch involves microelectrode
echniques, cell culture, video
icroscopy and digital image pro-
essing. Experience and aptitude
at least one of these areas is es-
ential. Additional experience in
he biochemistry of proteases and
rotease inhibitors would be an
dvantage. Salary will be on the
R1A scale (£11,399 — £18,165).
pplications consisting of a cur-
iculum vitae, research experience
nd the names of two academic re-
erees should be sent to **Dr. R. R.
ibchester, Department of
hysiology, University Medical
chool, Teviot Place, Edin-
urgh EH8 9AG. Ref. No. 5862.**

(6074)E

Postdoctoral Fellowship Position

Immunology/Molecular Biology/Biochemistry

Genentech, Inc., a leader in the biotechnology industry, has an outstanding postdoctoral opportunity within our Inflammation and Connective Tissue Research Laboratory.

This research investigates cellular interactions in chronic inflammation and tissue remodeling. We seek a highly motivated individual to join a group exploring T lymphocyte specificity at sites of chronic immune-mediated inflammation. Studies are directed toward delineating T cell antigen receptor usage, control of lymphokine production and T lymphocyte-fibroblast interactions.

Candidates should have a PhD in Molecular Biology, Immunology or Biochemistry and be well versed in molecular biological techniques.

Our progressive company offers an extensive benefits package which includes fully paid dental/medical/vision coverage for you and your dependents, child care facilities, free health club membership and an employee stock purchase plan. For immediate consideration, please send your CV as well as the names and addresses of three references to Edward P. Amento, Genentech, Inc., 460 Point San Bruno Blvd., South San Francisco, CA 94080. We promote and actively support affirmative action and equal employment opportunity. Women and minorities are encouraged to apply.

Genentech, Inc.

(NW6643)E

King's College School of Medicine and Dentistry of King's College London

JOINT RESEARCH COMMITTEE PhD STUDENTSHIPS

King's is pleased to announce the establishment of an additional 3 PhD studentships which will be available this year at it's School of Medicine and Dentistry in Bessemer Road, London SE5.

These studentships will be supported by the Joint Research Committee of Camberwell Health Authority and King's College School of Medicine and Dentistry, and will be established in respect of nominated research projects. Each studentship award will provide support at the level offered by the SERC.

Full details of the projects to be supported by these Studentships will be placed in the June/July issues to this publication, and will include details of how to apply.

Contact: Ms. K. Quantrell, 071-326 3557. (6067)F

UNIVERSITY COLLEGE LONDON

MEDICAL MOLECULAR BIOLOGY UNIT

Department of Biochemistry & Molecular Biology

RESEARCH STUDENTSHIP

A research studentship, funded for three years by the AFRC, is available to study the function of members of the transforming growth factor-β gene family in the developing chick limb. The project will give training in the experimental manipulation of chick embryos and in a broad range of molecular biological techniques, including the use of retroviral vector systems to deliver genes to embryonic chick tissues.

The successful applicant will work under the supervision of Dr P Brickell and will join a team studying pattern formation in the chick limb and face, in collaboration with Dr C Tickle and Professor L Wolpert of the Department of Anatomy and Developmental Biology.

Applicants should send their curriculum vitae and the names of two referees to Dr P Brickell, Medical Molecular Biology Unit, The Windeyer Building, Cleveland Street, London W1P 6DB. Further details can be obtained from the same address.

Closing date for applications: 27th May, 1991. (6108)F

POSTDOCTORAL FELLOWSHIP UNIVERSITY OF MASSACHUSETTS MEDICAL SCHOOL

Applications are invited for a postdoctoral position as an Associate to work in the laboratory of Dr Roger J Davis. The successful candidate will join a dynamic group of scientists and participate in an exciting research environment. Our research is focused on the examination of the mechanism of signal transduction by the EGF receptor. Qualified candidates should possess either a MD or PhD in the biological sciences. Preference will be given to individuals with experience in molecular biology or protein chemistry.

Please forward your CV and three letters of recommendation to: Dr Roger Davis, Program in Molecular Medicine, University of Massachusetts Medical School, 373 Plantation St, Worcester, MA 01605. An Equal Opportunity Employer.

(NW6642)E

RESEARCH STUDENTSHIPS

SCHOOL OF PHARMACY ***UNIVERSITY OF LONDON***

Applications are invited from students who have or expect to obtain a first or upper second class honours degree in Biochemistry, Pharmacology or related discipline for two Research Studentships in the laboratory of Dr F A Stephenson. One of these is a CASE award with a major European Pharmaceutical Company. The successful candidates will join a newly established active research group whose interests centre on the structure, function and regulation of neurotransmitter receptors, most notably GABA and glutamate receptors. The students will receive training in a variety of techniques pertinent to the study of these important brain proteins, and these will include radioligand binding studies, receptor isolation, site-directed antibody production and molecular biological methods.

Applicants should send two copies of their curriculum vitae with the names of two academic referees to **Dr F A Stephenson, Department of Pharmaceutical Chemistry, School of Pharmacy, 29/39 Brunswick Square, London WC1N 1AX,** (to whom informal telephone enquiries can be made. Tel. 071-753 5877). (6091)F

FELLOWSHIPS/STUDENTSHIPS continued

UNIVERSITY OF LEICESTER

Department of Physiology

MRC STUDENTSHIP

Applications are invited for an MRC studentship to study for a PhD for 3 years from 1 October 1991. The student will undertake a research project as a member of one of the following research groups:

the ion channel group, using patch clamp to study ion channels in smooth muscle, skeletal muscle and neurones;

the membrane transport group, studying active transport mechanisms, excitation-contraction coupling in heart and control of cellular volume in kidney and brain;

the neurophysiology group, studying muscle mechanoreceptors, nerve regeneration and autonomic neurotransmission.

Informal enquiries should be made to Peter Stanfield or John Tunstall, postgraduate tutor (Tel 0533 523300 or 523093) from whom a booklet detailing projects is available. Applications including a cv and the names of two academic referees, should be sent to **Professor P R Stanfield ScD, Department of Physiology, University of Leicester, PO Box 138, Leicester LE1 9HN.** (6081)F

UNIVERSITY OF LIVERPOOL

Department of Human Anatomy and Cell Biology

RESEARCH STUDENTSHIP

funded by

YAMANOUCHI PHARMACEUTICAL CO LTD

Applications are invited for a research studentship, leading to the award of PhD, to study the effects of actin-binding peptides on contractility, ion balance and ATP levels in single heart muscle cells. The project involves the use of bioluminescent intracellular probes (for Ca^{2+}, ATP), fluorescent probes (Na^+, pH) and computer-based video measurements of cell contratility. The work will be conducted in the Single Cell Group under the supervision of Professor Peter Cobbold. The Group has c.10 members; two experienced, post-doctoral research assistants will be involved in the project. The value of the studentship is £8,000 pa rising to £9,245 in year 3. Fees will also be paid by Yamanouchi Pharmaceutical Co Ltd.

The studentship, which starts in October 1991, is available to UK nationals only. Applicants who have, or expect to obtain, at least a 2(i) honours degree in a biological subject should apply promptly enclosing a CV and the name, address and telephone or fax numbers of two academic referees to: **Professor P H Cobbold, Department of Human Anatomy and Cell Biology, The University, PO Box 147, Liverpool L69 3BX. Tel: 051-794 5470 Fax: 051-794 5517.**

AN EQUAL OPPORTUNITY EMPLOYER (6083)F

DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY
UNIVERSITY OF NOTTINGHAM MEDICAL SCHOOL

POSTGRADUATE RESEARCH STUDENTSHIPS

Applications are invited from those who have or expect to obtain a good Honours Degree (or equivalent) in Pharmacology, Physiology or a related subject for 7 three-year research projects which will commence in October 1990 in the following areas: MRC Collaborative Awards: Distribution and Function of H_3 receptors in cultured cells (Dr S J Hill); Excitatory amino acid receptor-stimulated second messenger formation in brain (Dr D A Kendall); Changes in serotonergic function with age (Professor C A Marsden). Glaxo Group Research Studentship: Effect of β-adrenoceptor agonists on intracellular second messenger formation in cultured cells (Dr S J Hill). AFRC Studentship: Visual entrainment of the circadian clock: from neurotransmitter signal to gene expression (Dr R Mason). One SERC CASE and one MRC Quota award in Neuropharmacology, Cardiac and Regional Haemodynamics, Human Metabolism and Nutrition or Drug Development and Toxicology. **Applicants should send a full curriculum vitae, including the names and addresses of two academic referees, to the individual named with the project, or to Professor T Bennett, Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH, indicating the area of your interest.** (6073)F

continued on p18

COURSES

EMBO PRACTICAL COURSE

MAKING ANTIBODIES IN BACTERIA AND ON PHAGE

September 28 — October 6, 1991

at IRBM Pomezia (Rome) — Italy

Organized by: Giovanni Cesareni; Riccardo Cortese; Giovanni Galfré and Gregory Winter

***Instructors:* from IRBM**
Riccardo Cortese, Franco Felici, Armin Lahm, Maurizio Sollazzo, Andreas Crameri, Giovanni Galfrē, Alessandra Luzzago, Anna Tramontano

— from the 2^D University of Rome — Tor Vergata
Luisa Castagnoli, Roberto Jappelli, Giovanni Cesareni

— from Cambridge Center for Protein Engineering and LMB
Gregory Winter

— from Cambridge Center for Protein Engineering
Michael Baier, Phillip Holliger, James Marks, Andrew Griffiths, Hennie Hoogenboom

— from LMB, Cambridge
Peter Jones

New technologies offer fresh ways of making antibodies and refining their properties — see Winter and Milstein "Man-made antibodies", Nature 349, 293–299 (1991).

The Course will focus on:

1. PCR cloning of antibody genes;
2. expression of soluble antibody fragments in bacteria;
3. assembly of repertoires of antibody genes from immunised animals and their expression on the surface of bacteriophage;
4. selection of phage and antibody genes by affinity chromatography.

There will also be lectures covering further aspects of antibo engineering and phage display technologies.

Vectors, primers and protocols will be distributed for the participants take home.

The Course will be limited to 14 participants from European Resear Institutions. Preferred applicants will be at the advanced predoctoral or t postdoctoral stage. Candidates must send a curriculum-vitae and a conci statement of the interest in this Course as related to their research projec Participation, including board and lodging, will be free of charge. No fun are available to cover travel expenses. **Send your application within Ju 15, 1991 to: Prof. Riccardo Cortese, IRBM, Via Pontina km. 30,60 00040 Pomezia (RM), Italy. Fax 39–6–91093654** (W8311)D

ECACC

School of Biological Sciences
European Collection of Animal Cell Cultures
PHLS/CAMR
and School of
Biological Sciences

UNIVERSITY OF SURREY

Advanced Techniques in Animal Cell Culture

8-13 September, 1991

A joint five-day training course of seminars and practicals, to be held at th University of Surrey, Guildford. The programme will include speakers from bot Departments plus a panel of invited specialist guest speakers from Research an Biotechnology Institutes.

The topics to be covered will include Cell Culture Techniques, Hybridom Technology, Growth Factors, Quality Control Methods, Cell Banking Cytogenetics, Biochemical Analysis and Scale-up Technology.

The Course is aimed at persons already working in a cell culture environment wh wish to extend or update their expertise in these selected topics. Practicals ar designed to provide 'hands on' experience for the participants.

Registration is limited to 30 and the course fee of £890 will include meals an accommodation for five nights.

For further details and an application form, contact Mrs M. Hodson School of Biological Sciences, University of Surrey, Guildford GU2 5XH Tel. 0483 571281 Ext. 2662. (6089)D

TENDERS

MAFF Ministry of Agriculture Fisheries and Food

OPEN CONTRACTING SCHEME 1992/93

The Ministry of Agriculture, Fisheries and Food intends to commission research projects in the areas listed below.

Proposals are invited for short-term R&D projects (1-3 years) on the following topics to begin in 1992/93.

A. Reduction in nitrogen inputs as a factor affecting plant physiology and development of pests and diseases.
B. Pesticide resistance in peach-potato aphid: genetic factors associated with variable overwintering success on oilseed rape and other hosts.
C. Companion cropping to enhance the implementation of integrated pest control.
D. Review of microbiological processing of agricultural and forest products.
E. Glutathione-mediated responses of plants to stress.
F. Polymerase chain reaction and luminescence techniques to improve the protection of crops against plant pathogens.
G. Vesicular/arbuscular mycorrhizal fungi as biological control agents on horticultural crops.
H. Measurement of gaseous emissions (particularly ammonia) from soils, farm wastes and farming systems.
I. Modelling leaching (especially of nutrients and pesticides) from cracking clay soils.
J. Nitrification/denitrification processes in soils and surface waters (including ditches and drains) and release of nitrogen to atmosphere, especially as nitrous oxide or ammonia.
K. Climate change and water/nutrient relationships between plants and soils (involving roots and mycorrhiza).
L. Impact/benefit Assessment:
(i) To devise a framework for the assessment of costs and benefits, especially of externalities, arising from pesticide usage.
(ii) Environmental impact/benefit assessment of changing farming practice.
M. Crop Management and Storage in Environmentally Friendly Farming:
(i) Implications of changing agronomic practices (eg in low input and organic systems, use of plastic coverings, early harvesting) for associated pesticide residues and mycotoxins.
(ii) Alternative storage practices (eg avoiding the use of sprout suppressants or post harvest pesticide treatments).
(iii) Assessment of potentially harmful effects from the use of natural crop protectants (eg pest or disease resistance factors in "naturally resistant crops").
N. Significance of somatic cell counts in sheep's and goats' milk.
O. Microbiological aspects of poultry abattoir hygiene.
P. To find a safe alternative to sheep dipping which minimises residues and withdrawal periods as well as operator hazard and risk to the environment.
Q. Transmissible Spongiform Encephalopathies:
(i) Novel approaches to development of a diagnostic test for scrapie in live sheep.
(ii) Novel approaches to the aetiology of BSE or scrapie.
R. Assessment of welfare problems in housing systems for dry sows.
S. Survival of pathogenic organisms from farm wastes and fallen animals.

Applications with an element of industrial collaboration, where relevant, are particularly welcome.
Application forms and further details on the topic areas are available from: Mr Jonathan Alltimes, Ministry of Agriculture, Fisheries and Food, Room G15, Nobel House, 17 Smith Square, London SW1P 3JR (Tel: 071-238 5511).
Closing date: 14th June 1991.

(6101)T

AWARDS

THE ORAL AND DENTAL RESEARCH TRUST

PROCTER & GAMBLE RESEARCH AWARDS

ıe aim of these awards will be to provide funds to facilitate research in an tablished unit in the UK.

pplications are invited from graduates already working in oral and dental search, particularly those interested in research related to plaque, gingitis, caries, calculus and tooth hypersensitivity.

pplicants need not be graduates in dentistry and there is no limit of age, ne since graduation or nationality. Consideration will be given to applicaons from research workers from overseas participating in joint work in the nited Kingdom. The sum available for distribution in 1991-1992 is 15,000.

he closing date is 1st June 1991. There is no application form and applicants ould set out the research programme in which they are engaged and the urpose for which funds are required with detail costing, together with a pporting letter from their head of department or supervisor. Four copies each should be submitted. Applicants called for interview during June 991 may apply for reimbursement of reasonable travelling expenses.

is anticipated that the awards will take effect from the start of the 1991/92 ademic year.

pplications to: The Director, The Oral and Dental Research Trust, eats' House, 26 St Thomas Street, London SE1 9RN.

rocter & Gamble are the makers of Crest.

(6082)N

COURSES

CLINICAL MANAGEMENT OF STROKE

3rd/4th June 1991, London

The course will discuss the whole spectrum of clinical stroke, current approaches to its management, drug strategies that have been tried in the past and novel pharmacological approaches currently under development. The course will emphasise management, but will cover the basic essentials to understand the problems of stroke. A unique feature will be the opportunity for delegates to question stroke patients on the difficulties involved in their day to day care.

Speakers will be:
F. Clifford Rose, J. P. H. Wade, T. J. Steiner, J. de Belleroche (Charing Cross, UK), J. W. Norris (Toronto), W. Pryse-Phillips (Newfoundland), D. J. Thomas (St. Mary's, UK), R. Langton-Hewar (Frenchay, UK), J. Belch (Ninewells, UK), D. Dunbabin (Western General, Edinburgh).

Details from: **Georgina Mason, IBC Technical Services Ltd., Bath House, 56 Holborn Viaduct, London EC1A 2EX.**
Tel. 071-2364080. Fax. 071-4890849.

(6093)D

continued on p19

nature

the widest international selection of jobs in science — EVERY WEEK

INTERFACIAL PHENOMENA

in the Environment

AN INTERNATIONAL CONFERENCE IN HONOUR OF WERNER STUMM

October 6-11, 1991 Davos, Switzerland

Invited speakers:
J.J. Morgan (Caltech), M. F. Hochella, Jr. (Stanford Univ.), J. Lyklema (Univ. of Wageningen), J. Westall (Oregon State Univ.), W. Norde (Univ. of Wageningen), H. van Leeuwen (Univ. of Wageningen), W. van Riemsdijk (Univ. of Wageningen), A. Baiker (ETH-Zürich), W. Stumm (EAWAG/ETH-Zürich), B. Sulzberger (EAWAG/ETH-Zürich), A. Stone (Johns Hopkins Univ.), J. Hering (EAWAG/ETH-Zürich), Ch. O'Melia (Johns Hopkins Univ.), J. Buffle (Univ. of Geneva), L. Sigg (EAWAG/ETH-Zürich), A.J.B. Zehnder (Univ. of Wageningen), N. J. Brewin (John Innes Centre for Plant Science), B. Schink (Univ. of Tübingen), H. van Gemerden (Univ. of Groningen), B. B. Jorgensen (Univ. of Aarhus), H. Jannasch (Woods Hole Oceanographic Inst.), J. Schnoor (Univ. of Iowa), B. Wehrli (EAWAG/ETH-Zürich), S. J. Eisenreich (Univ. of Minnesota), F. Morel (MIT), T. Rosswall (IGBP, Stockholm), J. Beer (EAWAG/ETH—Zürich).

Objectives:
To bring together an international group of young and established scientists from different disciplines to discuss interfacial phenomena in the environment. Emphasis is placed on chemical and microbial processes. Molecular as well as global aspects will be addressed. A special session will consider how to improve the transfer of knowledge from science to society.

Registration and accommodation
The Conference will be open to scientists from all over the world. Attendance will be limited to about 150 people, including speakers, discussion leaders, invited scientists and those selected among the applicants. Registration fee and accommodation will be charged to participants. Some grants will be available for young researchers and other participants in need of support; they include registration and accommodation fees but no travel support.

Participants are expected to stay for the whole conference. The costs will be Swiss francs 400.- for registration fee, and 800.- for accommodation, including the conference dinner but not the excursion. Full fee is charged regardless of the time a participant attends the conference. Accommodation has been reserved at the Steigenberger Belvédère Hotel, Davos.

Application
Application forms can be obtained at the Conference Secretariat, c/o Ms B. Schwertfeger, EAWAG, 8600 Dübendorf, Switzerland (Tel. 01 823 50 35).
The deadline for all applications is June 15, 1991.

The Conference is Organized by:
EERO: European Environmental Research Organization
EAWAG: Swiss Federal Institute for Water Resources and Water Pollution Control
ETH: Swiss Federal Institute of Technology

(W8429)C

SYMPOSIUM ON TRANSFORMING GROWTH FACTOR-β AND RELATED PROTEINS IN DEVELOPMENT

Iowa State University, Scheman Building, Ames, Iowa, USA on September 20-23, 1991.

An international symposium on the structure, function, and developmental role of members of the TGF-β family. SPEAKERS: Akhurst, Daniel, Dawid, Donahoe, Hoffman, Madri, Massagué, Matrisian, Moses, Roberts, Runyan, Wozney

Deadlines: Abstracts: 1 August 1991. Registration: 1 September 1991

Information and Registration Materials: **MCDB/ISU Symposia Series, 141 Bessey Hall, Iowa State University, Ames, IA 50011 USA. Tel. No. (515)294-7252; FAX No. (515)294-1337**

(NW6594)M

PRIZES

$5,000 PRIZE

A former NASA physicist is offering an award of $5,000 to the first person who can demonstrate that his concept of a space drive will not work. A strong physics background is required. **Please contact Prof James H Vance, 4 Rocky Hill Road, Amherst, NH 03031.**

(NW6651)U

ENDOTHELIM SYMPOSIU

NATIONAL HEART & LUNG INSTITU

18, 19 & 20 NOVEMBER 1991

This is an exciting and fast moving area of research that has se dramatic developments over the past two years, having far rea ing consequences in many areas of medical research.

TOPICS

Atherosclerosis, reperfusion injury, solid tumour microcirc tion, metastasis, angiogenesis, lung injury, nitric oxide, neu peptides, endothelin and adhesion molecules.

PARTICIPANTS

Dr A Ager (Manchester), Professor E. E. Anggard (London) Dr S. A. Archer (Minneapolis, USA), Professor D. R. Blake (London), Professor S. R. Bloom (London), Dr S. D. Brain (London), Dr P. Collins (London), Professor J. Denekamp (London), Dr G Fearns (London), Dr J. Giddings (Cardiff), Dr D. Haskard (London), Dr K. Honn (Detroit, USA), Professor O Hudlicka (Birmingham), Professor J. MacDermo (London), Professor J. Martin (London), Dr S. Moncada (Beckenham), Dr C. Packard (Glasgow), Dr J. D. Pearson (London), Professor J. Polak (London), Professor U. S. Ryan (St Louis, USA), Professor T. A. Springer (Boston, USA), Professor Sir John Vane (London), Dr J. B. Warren (London) Dr C. Wegner (Ridgefield, USA), Professor A. H. Weston (Manchester) Dr F. M. Williams (London), Professor T. J. Williams (London), Professor N. Woolf (London).

ORGANISERS

Dr John Warren and Professor Timothy Williams, Appli Pharmacology Department, National Heart and Lung Institu

FEE

For 3 days, including catering: £390. Academic rate £250

ENQUIRIES

Postgraduate Medical Centre, National Heart and Lu Institute, Dovehouse Street, London SW3 6LY, tel: 0 351 8172, Fax: 071 376 3442.

(6068)N

continued o

FELLOWSHIPS/STUDENTSHIPS continued

King Alfred's College
WINCHESTER

15
YEAR

POSTGRADUATE RESEARCH STUDENTSHIP IN BIOLOGICAL SCIENCES

A studentship commencing in September 1991 is available to work o ultrastructure, biophysics and secretion of the collagenous egg case o dogfish. The project has the following aims:

1. **To investigate the ultrastructure, anatomy and histochemist the nidamental gland to understand more about the formati the egg case.**
2. **To characterise the collagen and associated proteins secrete different regions of the nidamental gland to understand how interact in fibrillogenesis and the formation of this hi organized structure.**
3. **To investigate the possibility of in vitro studies on nidame gland tubules with the long term aim of investigating control dynamics of synthesis and secretion.**
4. **To investigate tthe biophysics of the egg case, relating structu function.**

The student will be expected to register for a higher degree. The stu may undertake up to six hours work a week as a college demonstr Letters of application should be sent by May 10th, 1991 to **Dr D P Kn Department of Biological Sciences, King Alfred's College, Spark Road, Winchester SO22 4NR.**

(607

---

nature — the professional choice

Royal Postgraduate Medical School

(University of London)

Monoclonal Antibody Techniques

Specialist Short Course

24-28 June 1991
18-22 November 1991

Applications are invited for this full-time one week course. The course consists of lectures and practicals covering the following topics:

- *Immunisation*
- *Choice of myeloma lines*
- *PEG cell fusion*
- *Electrofusion*
- *Cloning*
- *Screening systems*
- *Antibody purification*
- *Immuno-blotting*
- *The effects of affinity/avidity in different assay systems*
- *Epitope mapping and mimotope design*
- *Discussion on some of the current uses including antibody engineering and clinical applications*

Course organisers: M Ritter
H Ladyman
N Reza

Course fee (incl catering): **£380.00**

Further details from: Wolfson Conference Centre
Royal Postgraduate Medical School
Du Cane Road
London W12 0NN

Telephone: 081 740 3117
081 740 3245

Fax: 081 740 4950

(5863)D

KING'S COLLEGE LONDON

BIOMEDICAL SCIENCES DIVISION

POSTGRADUATE COURSES

The Biomedical Sciences Division invites applications for the 1991/92 session from graduates in relevant science subjects, pharmacy or medicine, for the following courses:

MSc in Biomedical Research — 1 year full-time or 2 years part-time, a preparation for a career in laboratory, clinical or industrial research. The course includes 1 CU of 3rd year undergraduate options, 1 CU advanced technical workshops, and a 6–9 month research project. Entry qualifications: degree or equivalent in a biomedical or related field. Suitable for new graduates, or mature students needing a higher qualification or retraining.

MSc in Human and Applied Physiology — A one year (two-year part-time) course involving the study and measurement of human function in normal and abnormal environments. This course is accepted by MRC for tenure of advanced course studentships.

MSc in Pharmacology — A one-year full-time course in General Pharmacology. This course is accepted by SERC for tenure of advance course studentships.

MSc in Research Methods for Remedial Therapists — A two-year part-time course which provides an introduction to the scientific principles of remedial therapy, intended for those possessing a qualification recognized by the CPSM and some professional experience.

Enquiries about any of these courses should be addressed to Professor S L Howell, Head, Biomedical Sciences Division, King's College London, Campden Hill Road, Kensington, London W8 7AH.

(6086)D

ANNOUNCING AN INTERNATIONAL IBC CONFERENCE

NEW DRUGS FROM NATURAL SOURCES

13th-14th June 1991 — GLOUCESTER HOTEL, LONDON

This conference brings together leading experts engaged in the search for new drug leads from natural sources, and will address: new drug sources, new drug targets and screening tools based upon isolated enzyme and receptor, and new screening methodologies suitable for high throughput testing of chemical and natural product sources. Examples will be discussed of the newly emerging products from such intensive novel high throughput targeted drug screening activities.

The meeting will be of interest to all scientists engaged in drug research and will provide a 'state-of-the-art' review of activities in the field of new drug screening.

Chairmen:

Dr Louis Nisbet, Xenova Ltd, UK
Dr Jim Coombes, Scientific Consultant, UK

Speakers:

Dr Tim Harris, Glaxo Group Research, UK
Dr Mary Napier, Genentech Inc, USA
Dr Manfred Wolff, ImmunoPharmaceutics Inc, USA
Dr Michael Dreyfuss, Sandoz Pharma Ltd, Switzerland
Dr Peter Schindler, Hoechst AG, Germany
Dr Jean Jacques Sanglier, Sandoz Pharma Ltd, Switzerland
Dr Wolfgang Weber, F Hoffman La-Roche, Switzerland
Dr Linda Fellows, Royal Botanic Gardens, UK
Professor David Kingston, Virginia Polytechnic and State University, USA
Dr Baruch Blumberg, Balliol College, UK
Professor Michael Fowler, University of Sheffield, UK
Dr Robert Hertzberg, SmithKline Beecham Pharmaceuticals, USA
Dr Robert McBurney, Cambridge Neurosciences, USA
Dr Paul Behrens, Martek Corporation, USA
Dr William Dower, Affymax Research Institute, USA
Dr Barry Berkowitz, Magainin Sciences, USA
Dr Louis Nisbet, Xenova Ltd, UK

For a full conference programme/registration details of the above event, please contact:

Catherine Barrett
IBC Technical Services Ltd
Bath House, 56 Holborn Viaduct,
London EC1A 2EX
Tel: 071-236 4080, Fax: 071-489 0849 (+44) (6103)C

INAUGURAL SCIENTIFIC SYMPOSIUM

WELLCOME TRUST CANCER RESEARCH CAMPAIGN

Institute of Cancer and Developmental Biology, Univerity of Cambridge

Cambridge England, 8 and 9 July, 1991.

SPEAKERS

B. Alberts, San Francisco	M. Dexter, Manchester	D. Melton, Cambridge MA
D. Baltimore, Boston	R. Hynes, Cambridge MA	C. Nusslein-Volhard, Tubingen
A. Colman, Birmingham	A. Klug, Cambridge, UK	J. Rossant, Toronto

Admission by registration only (free). Registration closes 14th June, 1991.

Limited College accommodation.

For further information and applicaton forms, apply to:
The Chairman, Wellcome CRC Institute, Tennis Court Road, Cambridge CB2 1QR, England. (6095)M

---

nature

Still first with the best in science

Concepts and Molecular Mechanisms of Multistage Carcinogenesis

First Joint Conference of the American Association for Cancer Research and the European Association for Cancer Research immediately following EACR-XI in Genoa

Additional Support from the Istituto Nazionale per la Ricerca sul Cancro

Grand Hotel Miramare, Santa Margherita, Italy
November 6-9, 1991

SCIENTIFIC PROGRAM COMMITTEE

AACR
I. BERNARD WEINSTEIN / New York, USA (Co-Chairperson)
ARTHUR P. GROLLMAN / Stony Brook, USA
HAROLD L. MOSES / Nashville, USA

EACR
PETER BANNASCH / Heidelberg, Germany (Co-Chairperson)
ALLAN BALMAIN / Glasgow, Scotland
JAN SVOBODA / Prague, Czechoslovakia

LOCAL ORGANIZING COMMITTEE

LEONARDO SANTI / Genoa, Italy (Chairperson)
MARIA COLNAGHI / Milan, Italy
MARGARET FOTI / Philadelphia, USA
CLAUDIO LOMBARDO / Genoa, Italy
MARCEL ROBERFROID / Brussels, Belgium

SCIENTIFIC PROGRAM

Welcoming Remarks
LEONARDO SANTI / Genoa, Italy

Opening Address
PETER BANNASCH / Heidelberg, Germany

Epidemiological and Experimental Evidence for the Multistage Process
HENRY C. PITOT / Madison, USA
ERICH HECKER / Heidelberg, Germany
FRANCO MERLETTI / Turin, Italy
FRIEDRICH MARKS / Heidelberg, Germany
FRANCIS V. CHISARI / La Jolla, USA

APPLICATION FORMS
(North and South America)
American Association for Cancer Research
Public Ledger Building, Suite 816
Sixth and Chestnut Streets
Philadelphia, PA 19106, USA
215-440-9300 215-440-9313 (FAX)

(Outside the Americas)
Italiana Congressi
Via Bensa 2
16124 Genoa, ITALY
10-202541 10-299382 (FAX)

DNA Replication, Damage, and Repair
MARCEL ROBERFROID / Brussels, Belgium
DEZIDER GRUNBERGER / New York, USA
HARTMUT M. RABES / Munich, Germany
ARTHUR P. GROLLMAN / Stony Brook, USA
PHILIP C. HANAWALT / Stanford, USA
TOMAS LINDAHL / Herts, England
SARASWATI SUKUMAR / San Diego, USA

Multiple Genetic Changes during Tumor Development
LANCE A. LIOTTA / Bethesda, USA
JAN SVOBODA / Prague, Czechoslovakia
ALLAN BALMAIN / Glasgow, Scotland
ANTON BERNS / Amsterdam, The Netherlands
TERRY H. RABBITTS / Cambridge, England
WEBSTER K. CAVENEE / Montreal, Canada

Genetic Aspects of Tumor Suppression
GEORGE KLEIN / Stockholm, Sweden
ERIC J. STANBRIDGE / Irvine, USA
MARY WEISS / Paris, France
DAVID P. LANE / Herts, England
NICHOLAS HASTIE / Edinburgh, Scotland
EDWARD HARLOW / Charlestown, USA

Growth Factors and Signal Transduction
HAROLD L. MOSES / Nashville, USA
GIUSEPPE DELLA PORTA / Milan, Italy
CHRISTOPHER J. MARSHALL / London, England
CARL-HENRIK HELDIN / Uppsala, Sweden
RIK DERYNCK / South San Francisco, USA

Overview and Future Directions
I. BERNARD WEINSTEIN / New York, USA

AACR EACR

The AACR and EACR invite the international community of cancer researchers to submit applications for this exciting conference by August 15, 1991.

Abstracts for consideration for poster presentation also due by August 15, 1991.

A limited number of travel grants for graduate and medical students, postdoctoral fellows, and physicians in training will be available.

(NW6656)C

Typesetting: Typedesign Ltd, London EC2 and J. W. Arrowsmith Ltd, Bristol. Printing: Chase Web Offset, Plymouth, a St Ives Company, and The Riverside Press Ltd, Whitstable, Kent. William Byr
Press Inc., Richmond, Virginia, USA and Obun Printing Co., Bunkyo-ku, Tokyo, Japan. Published by MACMILLAN MAGAZINES LTD, 4 Little Essex Street, London WC2R 3LF—25 April 1991.

Takeda

ONE STEP CLOSER

With its three billion genes, the human body is blessed with marvelously sophisticated—but virtually mysterious—mechanisms. Unlocking these enigmas is an extraordinary task but an imperative one, for it could lead to the discovery of new drugs. At Takeda, our team of dedicated research experts is progressing ever closer toward such discoveries.

TAKEDA

Takeda Chemical Industries, Ltd.

Head Office: 3-6, Doshomachi 2-chome, Chuo-ku, Osaka 541, Japan
Tokyo Head Office: 12-10, Nihonbashi 2-chome, Chuo-ku, Tokyo 103, Japan

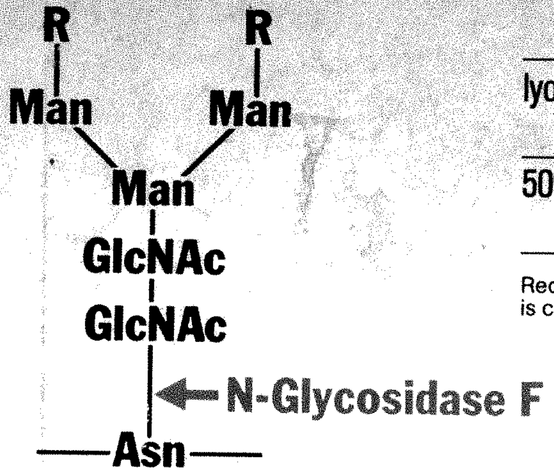
Reader Service No. 692

Boehringer Mannheim
Your source for glycoconjugate research products

NEW

recombinant N-Glycosidase F

(Endoglycosidase F-free. Highly purified from E. coli: no other glycosidases and proteases are detectable.)



lyophilisate:	Cat. No. 1365 185 - 20 units 1365 193 - 50 units
50% glycerol solution:	Cat. No. 1365 169 - 20 units 1365 177 - 50 units

Recombinant N-Glycosidase F
is cloned from Flavobacterium meningosepticum

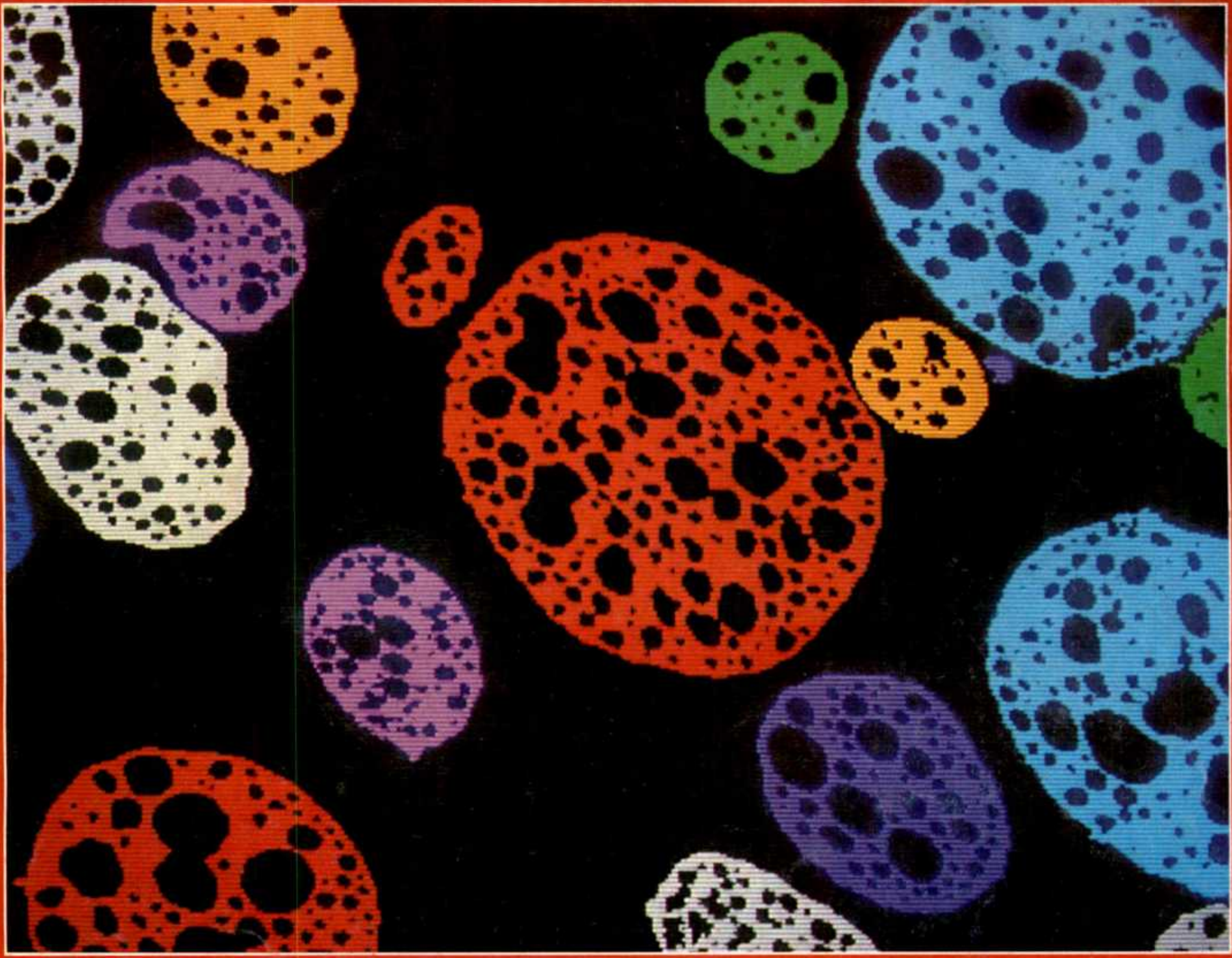
For further information on our Carbohydrate Analysis System please contact your local representative or

BOEHRINGER MANNHEIM

Boehringer Mannheim GmbH
Biochemica
P.O. Box 310120
D-6800 Mannheim 31
Reader Service No 11

nature

SUPPLEMENT TO VOLUME 350 NO. 6319, 18 APRIL 1991



ELF AQUITAINE: RESEARCH IN A LEADING INDUSTRIAL GROUP

REVIEW ARTICLE PROGRESS IN COLLOID SCIENCE

elf aquitaine

UK EDITION NOT FOR DISTRIBUTION IN THE USA

WHY IS APPLIED RESEARCH SO IMPORTANT TO YOU?

YOU MAY AS WELL ASK THE LIKES OF LUMIÈRE, PASTEUR AND EDISON.

At ELF AQUITAINE we take research very seriously. There are 5,700 researchers constantly working to expand frontiers in fields such as geology, chemistry, biology, medicine, pharmaceuticals and data processing. Among other innovations, our research has led to the development of revolutionary oil drilling techniques, new fuels, major discoveries in the treatment of cardiovascular diseases as well as products to replace CFCs and preserve the ozone layer. ELF AQUITAINE researchers are practical people, which is why they take their cue from the likes of Lumière, Pasteur and Edison, whose discoveries continue to have practical applications in everyday life. ■

elf aquitaine

PASSION IS OUR WISDOM.

BELIER

CUG - ~~H06912~~-

Nature is glad to publish this special supplement, the cost of which has been met by the French-based and petroleum-based multinational company Elf Aquitaine.

Our objectives are two-fold. First, to provide an opportunity for a research-based company to describe to regular Nature readers the diversity and intrinsic interest of the work on which it is engaged — in Elf's case this extends beyond oil exploration and production to petrochemicals, polymers and pharmaceuticals. Second, to provide readers with some material of a kind with which they are familiar, in this case a review article, Structures in Colloidal Physical Chemistry, for which there might otherwise not have been room in Nature.

That has an interest of its own. Petroleum, Elf Aquitaine's chief raw material, is used both in its better-known refined forms and in a variety of emulsified forms. Who would guess, for example, that lubricating grease is an emulsion of heavy-oil from distillation with soap? But the lesson chiefly to be learned from the article by Jacques Prost and Francis Rondelez on page 11 (which has been refereed in the usual way) is the degree to which the search for improved forms of petroleum-based materials now rests on (and has helped to stimulate) some of the most recondite of theoretical research — the study of the interactions between molecular aggregates as different as colloidal suspensions and liquid crystals.

John Maddox
EDITOR

contents

ELF AQUITAINE: RESEARCH AND DEVELOPMENT IN ONE OF EUROPE'S LEADING INDUSTRIAL COMPANIES
Claude Jablon — Page 2

ELF AQUITAINE AND THE ENVIRONMENT
Bernard Tremain — Page 4

ENVIRONMENTAL RESEARCH: THE EFFECTS OF AIR POLLUTION ON PLANTS
Jacques Bonte and Roger Camps — Page 4

IMAGE PROCESSING AS A TOOL IN INTERPRETING SEISMIC DATA
Naamen Keskes and Jaqueline Camy-Peyret — Page 6

ROCK MECHANICS IN OIL EXPLORATION AND PRODUCTION
Vincent Maury — Page 8

review article

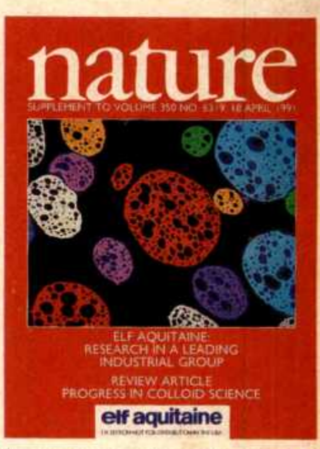
STRUCTURES IN COLLOIDAL PHYSICAL CHEMISTRY
Jacques Prost and Francis Rondelez — Page 11

A REFINER'S APPROACH TO ASPHALT CHARACTERIZATION
Helen King, Gayle King and Jean-Pascal Planche — Page 24

POLYMERIC PIEZOELECTRIC TRANSDUCERS
Richard A. Ferren — Page 26

POLYMER DESIGN FOR HIGH-PERFORMANCE MATERIALS: LIQUID-CRYSTAL POLYMERS
Didier Judas, Yves Germain, Claudine Biver, Sylvie Girault and Christiane Taupin — Page 28

CHEMICAL SYNTHESIS OF GLYCOSAMINOGLYCANS: NEW APPROACHES TO ANTITHROMBOTIC DRUGS
Maurice Petitou, Jean-Claude Lormeau and Jean Choay — Page 30



nature

SUPPLEMENT TO VOLUME 350 NO 6319 18 APRIL 1991

ELF AQUITAINE:
RESEARCH IN A LEADING
INDUSTRIAL GROUP

REVIEW ARTICLE
PROGRESS IN COLLOID SCIENCE

elf aquitaine

Image processing: structures in a biphasic polymer (high impact polystyrene) observed by transmission electron microscopy.
(Photo ATOCHEM)

ELF AQUITAINE: RESEARCH AND DEVELOPMENT IN ONE OF EUROPE'S LEADING INDUSTRIAL COMPANIES

Claude Jablon
Chief Scientist
Société nationale
elf aquitaine
Tour elf
92078 Paris La Défense
France

Elf Aquitaine is the largest quoted company in France and ranks in the top 20 in Europe with a turnover of 1,750,000 million French francs in 1990. It has its roots in the discovery in 1951 of a major natural gas field at Lacq in South-West France, and has since grown through acquisitions and industrial development. Here the company's chief scientist Claude Jablon describes Elf Aquitaine and its commitment to research and development.

Elf Aquitaine employs around 90,000 people worldwide in three lines of business - oil and gas, chemicals and pharmaceuticals.

OIL AND GAS

Elf Aquitaine's fields are located mainly in Africa and the North Sea but the company also has a presence in the Soviet Union, the Middle East and the Western hemisphere. It is very active in the refining market both in France and abroad, producing market leading products such as lubricants and special bitumen.

CHEMICALS

Elf's chemicals division, **Atochem,** has a very wide range of activities from basic chemicals, such as fertilizers and chlorine, to intermediate and speciality products. It is especially strong in sulphur and fluorine derivatives and produces a large range of polymers such as PVC, PS, PVDF, polyolefins, polyamides, and polymethylacrylates.

PHARMACEUTICAL AND RELATED PRODUCTS

Elf's pharmaceutical company **Sanofi** produces a range of pharmaceuticals which includes well-known cardio-vascular and nervous system drugs and diagnostic kits. In biotechnology, it is the world leader in gelatin, and is a major manufacturer of food additives and aromas. The company is developing a range of cosmetics.

In 1991 Elf Aquitaine will spend 4,282 million French francs on research and development. There are 5,600 R&D staff, of whom more than 2,000 are graduates. There is a wide range of activities and the R&D organisation is largely decentralised. Although Elf does not have a central research laboratory there is a sizeable central budget for long-term, high-risk projects, technologies which cut across divisions (such as computing, applied mathematics, chemical engineering and analytical techniques) and for cooperation with publicly funded research programmes.

ELF: EXCELLENCE IN SCIENCE AND TECHNOLOGY

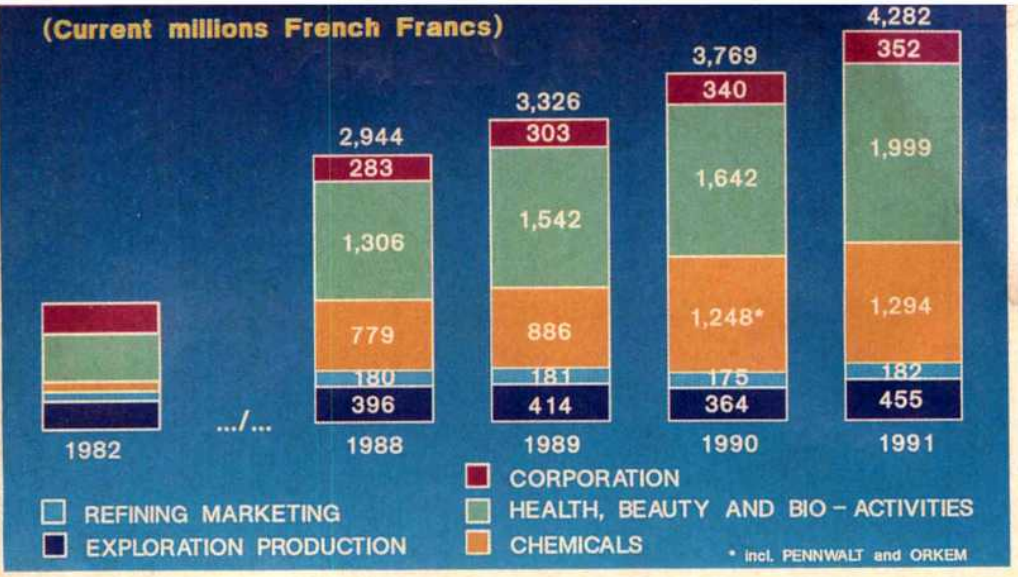
Elf Aquitaine was founded on excellence in science and technology. The gas field discovered at Lacq in 1951 was nearly passed over because at the time experts argued that the high sulphur and carbonic acid content made it uneconomical. Nevertheless the field was successfully developed, generating revenue for future growth and putting R&D firmly at the centre of Elf Aquitaine's corporate organisation and culture.

Today, the Elf research portfolio boasts a wide range of technologies and products in which the company is a world leader.

In exploration and production, Elf pioneered advanced techniques such as the use of automatic subsea equipment and horizontal drilling (which was recently used in the exploitation of the Zuidwal gas field in the Dutch Waddensee). The company has also invested heavily in research on rock mechanics, giving it an in-depth understanding of drilling and borehole stability and the use of advanced mathematical techniques for interpreting seismic data. Both these topics are discussed elsewhere in the supplement by the scientists working on them.

In refining, Elf is a leader in clean fuel technology and advanced marine lubricants and has developed a market-leading polymer-linked bitumen called Styrelf. The company's bitumen research is described on page 24.

Elf's basic chemical company, Atochem has a broad product range: it is a major producer of hydrogen peroxide, hydrazine, sulphur and sulphur compounds, and of halogen derivatives from organic solvents to CFC substitutes and bromine-based fire fighting products. Elf is also one of the market leaders in polymers such as PVC and polyolefins, with brand names such as Rilsan (polyamid 11 and 12), Altulor (PMMA) and Kynar (PVDF).



Elf Aquitane: growth in research and development spending

Atochem also manufactures fertilisers, as does Elf's US subsidiary Texasgulf, a phosphate producer.

Elf's pharmaceutical arm, Sanofi Corporation is widely known for its cardiovascular and nervous system drugs. Other recent successes include the introduction of an Elisa AIDS detection kit and of low-molecular-weight heparin. The development of the low-molecular-weight Heparin and the discovery of an antithrombotic pentasaccharide is described by the scientists concerned on page 30.

Sanofi is the leading producer of gelatin and among the major producers of polysaccharide food additives. In cosmetics, Roger & Gallet, Nina Ricci and Yves Rocher are its most famous brand names.

Elf has maintained its interest in advanced industrial processes that was sparked by the sour-gas treatment developed in exploiting the Lacq field. Its biological clean-up technique was used after the Exxon Valdez oil spill in Alaska and it has also developed large scale industrial photochemical processes, techniques for heavy oil conversion and is currently working on methane conversion.

But Elf's technical prowess is not just directed inwards. Its recent research in organic geochemistry established the connection between heavy oil compounds and biological cell membranes, and led to the development of tools for dating Mesopotamian bitumen samples.

One of the company's geophysicists developed a new kind of signal transformer for seismic signals, which has recently opened up research on wavelet transformations.

Elf's long list of success stories is due to the dedication of its staff and the management commitment to technology. Much of the work is based on cooperation with universities.

COOPERATION WITH UNIVERSITIES

Science is the driving force of every serious technological company, but harnessing basic science presents a major challenge: basic research is costly and its contribution to company profits is not immediately obvious.

Elf's approach is to set up collaborations with public research institutes around the world, giving it access to emerging technologies, scientific support for its own research programmes and providing a training ground for future research staff.

Cooperation works through several channels, from simple contracts for the use of expensive advanced equipment to the setting up of joint research teams with the French National Centre for Scientific Research (CNRS). Elf currently funds about 600 collaborations both in France and abroad.

Particular emphasis is given to international staff exchanges; there are now more than 30 PhD students and post doctoral fellows sponsored by Elf in Europe and North America and 20 post doctoral fellows from the United States, UK and Germany are sponsored in French university laboratories. As Europe becomes more united, such exchanges will become more common.

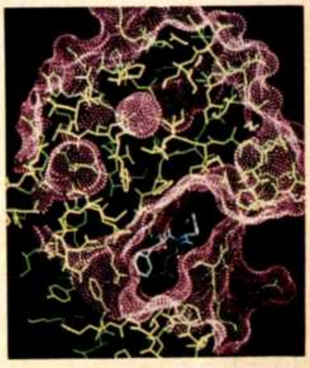
PRESENT AND FUTURE ISSUES

All large corporations face thorny problems in the areas of human resources, environmental protection and ethical issues.

Skills shortages will persist — especially if graduates in Europe follow the North American trend and reject technical careers. Elf is countering this by promoting positive images of science and technology careers through its relationships with higher education and by promoting a better understanding of the importance of science and technology.

Although dealing with skills shortages and environmental and ethical problems depends in part on political decisions, Elf believes that it can contribute to their solution through technical progress, involvement of its staff and by building relationships with local, national and international institutions.

Through its high level commitment to science and technology Elf Aquitaine is helping to solve these problems, and at the same time preserving the values on which our common culture is grounded.



ELF AQUITAINE AND THE ENVIRONMENT

Bernard Tramier
Director of
Environmental Affairs
Elf Aquitaine
Roger Camps
Environment Division
Elf Aquitaine
Jacques Bonte
CDERE, Lagor
64150 Moureiux
France

In this article Bernard Tramier, Director of Environmental Affairs outlines the company's approach to environmental protection and below two researchers describe a project carried out by Elf in collaboration with the French environmental studies institute CDERE (Centre Departemental d'Etudes et de recherches sur l'Environnement).

Environmental protection has been central to Elf Aquitaine's strategy ever since the exploitation of the Lacq natural gas field in 1951.

A number of technical developments were needed to protect the environment before the gas at Lacq, rich in hydrogen sulphide, could be exploited. As a result of this experience, Elf has always been committed to ensuring that growth is not achieved at the expense of the environment.

Today, more than 500 staff work on environmental issues, making environmental protection a field of Elf expertise in its own right. These staff make regular contributions to the work of international organisations such as the United Nations Environment Programme and the specialist commissions attached to France's Department of the Environment.

In recent years, Elf's approach to pollution control has progressed from cleaning up air and water discharges by installing scrubbers and other purification devices downstream of the production process, to developing clean technologies which do not generate pollution in the first place.

Clean-up techniques continue to be important, but the clean technology approach is now mandatory, and environmental protection features are designed in from the start of any new projects. Engineers check each stage in the development of products and processes to minimise environmental effects still further, through energy savings, reduced emissions and optimized production processes.

Preventing pollution accidents is a major concern throughout the company. Strict procedures are applied at all times and at all stages of industrial processes, from the building of plant through to control, maintenance and continuous monitoring of operations. Staff training and in-house environmental audits are essential components in achieving this objective.

The company also communicates and cooperates widely with public bodies on environmental protection. In that spirit Elf Aquitaine signed an agreement with the French Federation of National Parks (Fédération des Parcs Naturels de France) to promote nature conservation. Each year 3 parks receive a grant for an education project selected by a programme committee.

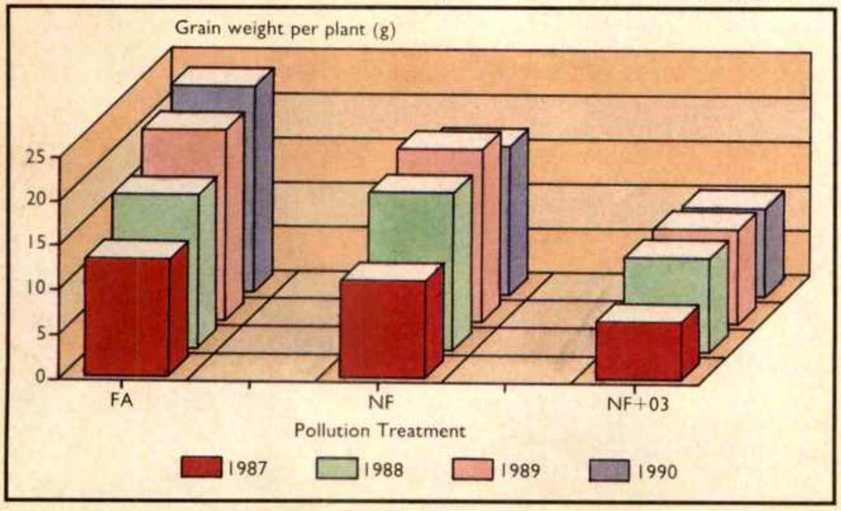
Elf Aquitaine has a dedicated budget for environmental research in areas such as offshore pollution, waste water treatment, atmospheric emissions, ecotoxicology, waste disposal and risk analysis.

These research programmes are conducted both in Elf's own labs and in collaboration with external public and private research centres. So far, Elf has funded more than 40 PhDs theses on environmental topics.

Although Elf's in-house requirements take priority in this research, there have been a number of spin-offs including the Sulfreen desulphurisation process, a rotating oil drum skimmer and a biodegradation acceleration agent Inipol EAP 22, which was successfully used in cleaning of Alaskan beaches following the Exxon Valdez oil spill.

The research described below is a typical example of a collaboration between Elf and a public research institute, CDERE.

Figure 1: Exposure-Response Relationship with Beans (*Phaseolus vulgaris*) grown in Open-Top Chambers



ELF RESEARCH INTO THE EFFECTS OF AIR POLLUTION ON PLANTS

In the past 15 years there has been a significant change in the nature of air pollution in Europe. While local, acute pollution has practically been eradicated, there have been large-scale increases in levels of atmospheric sulphate and nitrogen compounds and widespread acid rain.

A number of cases of ozone pollution have been monitored in which concentrations in plants have widely exceeded expected levels. Overall, there has been a 15 ppb increase in this type of pollution in the past 20 years with a forecast of 1-2% annual increase during the next few years.

In the early 1980s, previously unrecorded kinds of damage to forests in Western Europe was attributed to this pollution. Although in North America forests were virtually unscathed, the North East USA and Canada reported serious problems with lake acidification.

In Europe a number of national programmes were set up to study the causes of forest decline while in the US the National Crop Loss Assessment Network (NCLAN) showed that ambient ozone concentrations were seriously eroding productivity. In 1986 the European Community set up a research programme on the NCLAN model, bringing together 13 research institutes to investigate the effects of pollution on agricultural productivity in Europe.

At the same time techniques for studying the effects of air pollution have become much more sophisticated. Some work is still done in laboratory phytotrons, but dose-effect relationships are now investigated in open-top chambers to closely mimic natural conditions.

The open-top chamber consists of a cylindrical frame covered in polythene. Ambient air is scrubbed by a charcoal filter before being blown into the chamber via a pipe positioned 10 centimetres above the plant canopy.

Computer-controlled pollution generators then create atmospheres in which pollutant concentration can be strictly controlled.

An experiment using these techniques has been in progress for five years in the French Pyrenees Atlantiques area with Elf collaborating with CDERE under the auspices of the European Community open top chamber and French Deforpa forestry programme.

EFFECTS OF AMBIENT AIR POLLUTION ON CROP YIELD

The aim of this study is to assess crop losses due to air pollution and establish precise dose-effect relationships on yields. Three types of experiment are used:

1. Filtered air (FA) is blown into the chamber through the charcoal filter, reducing sulphur dioxide pollution and cutting ozone by 80%;
2. Non-filtered air (NF) is blown into the chamber and the plants are subject to natural ozone fluctuations;
3. Non-filtered air and ozone (NF+0_3): 20 ppb extra ozone is added to ambient air between 10am and 5pm GMT.

The experiment is strongly influenced by the weather conditions so it must be repeated for several seasons. Ambient ozone levels vary from one year to another: in 1987, 1989 and 1990 the summers were sunny with high ozone levels, in 1988 the summer was wet and the ozone level relatively low.

There were no ozone injuries on plants in the filtered air chambers, whereas ozone injuries occurred on all plants in non-filtered chambers. Injuries were much worse in the NF+0_3 chamber and the same symptoms occurred on crops in local fields after pollution episodes.

At harvest there was a strong correlation between productivity and ozone concentration.

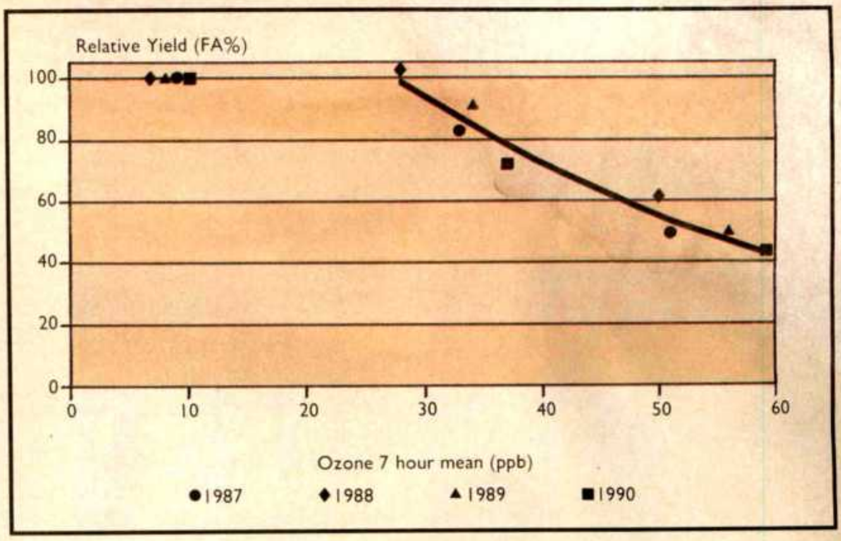
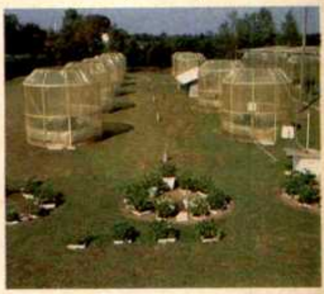


Figure 2: Effects of ozone on yield of Beans (*Phaseolus vulgaris*) grown in Open-Top Chambers (below)



Laboratory studies also revealed that leaf stomata did not close properly when the plant was subject to ozone stress.

These results will now be compared with those of the other EC investigations.

EFFECTS OF OZONE AND SULPHUR DIOXIDE ON NORWAY SPRUCE

In another study Elf scientists have spent five years looking at the direct effects of ozone and sulphur dioxide on Norway spruce (*Picea abies*).

Air pollution levels at the Donon Pass in the Vosges area of France, where severe dieback of Norway spruce has occurred, were replicated in the experimental chambers.

The tops of the chambers were closed when it rained to limit the study to the effects of air pollution rather than rainwater pollution.

Three clones of *Picea abies* from Poland, West Germany and France were studied under six conditions: ambient air, unfiltered air, filtered air plus $S0_2$, filtered air plus 0_3, filtered air plus $S0_2$ and 0_3; and filtered air.

After five years no significant difference in growth (height, trunk diameter, needle weight and length, growth rate, growth stages and yearly shoot increments) was observed. There was some yellowing and early shedding of older needles in the French clone during the last two years of the experiment, but this was the only example of the type of ozone damage that is seen on broad leaved plants and crops. It was concluded that the Norway spruce is very resistant to air pollution.

Although there was some variation in response to air pollution between trees from different locations, the classic symptoms of forest decline that have been blamed on air pollution were not seen. The results indicate that ozone alone is not to blame for the decline, but that in combination with natural stresses, air pollution can damage plants.

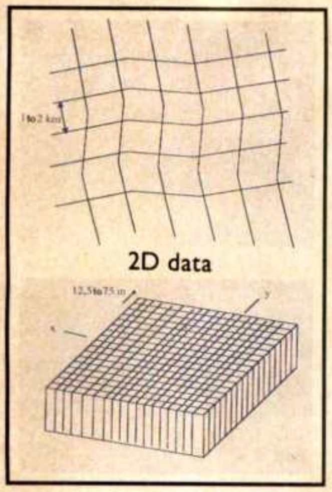
The Elf Aquitaine/CDERE cooperation will investigate possible synergy between different stress factors such as ozone pollution, drought and nutrient deficiency.

IMAGE PROCESSING AS A TOOL FOR INTERPRETING 3D AND 2D SEISMIC DATA

Naamen Keskes and
Jaqueline Camy-Peyret
SNEA(P)-CSTJF 64018
Avenue Larribau
Pau
France

This article examines the application of computerised image processing techniques to the interpretation of 3D and 2D seismic data in oil exploration and production.

Reflection seismology is the most important technique for determining deep geological structure. 2D data, used for regional reconnaisance and initial assessment of exploration potential, consists of vertical profiles or traverses taken from hundred to thousands of metres apart, with two orthogonal directions of acquisition for aligning profiles. 3D data, used for detailed assessments of known oil fields or in exploration areas where the presence of oil has already been confirmed, is built up from regularly spaced soundings along *x* and *y* axes and depth (see Figure 1). In both cases, the results are presented as maps of all seismic markers (commonly referred to as horizons).



2D data

3D data

Figure 1

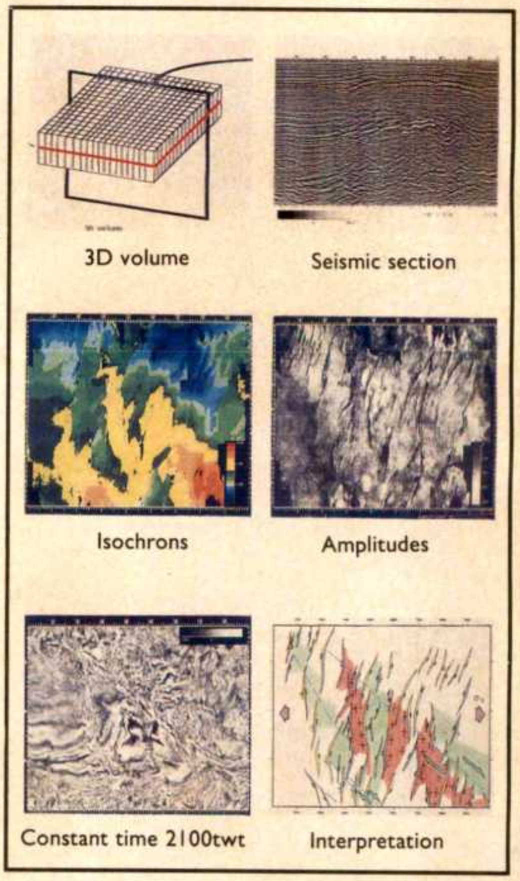
Determination of the subsurface fault pattern is one of the crucial steps in interpreting 2D and 3D seismic data. Precise identification of the different types of faults and their relationship to the sedimentary layers they intersect is an essential element of the overall geological model that will be used to assess oil trapping structures.

As part of its Sismage research project, Elf Aquitaine has developed a software application, called SURFACES, for interpreting large volumes of seismic data (Figure 2). Surfaces also incorporates new methods of validating the interpretation.

SURFACES analyses data in two stages:

1. Bulk interpretation - rather than attempting to follow a specific seismic marker throughout the 3D survey, SURFACES interprets all events in one pass. The result is a number of elementary horizons, the boundaries of which are defined by discontinuities in tracking consistency.
2. Assembly - the interpreter assigns elementary horizons, or surfaces, to a specific geological marker. The interpreter is supplied with the spatial representation of a surface plus other geophysical attributes, as decision support factors for including surfaces in a marker. Using pattern matching, the interpreter can chose to accept or reject a surface during assembly. The most useful attribute in surface assembly is the amplitude of the surface.

During the interpretation of a marker over the whole survey, both depth and attribute maps are used to determine the major faults (based on significant discontinuities) and minor faults, lineaments and flexures (using illuminated attribute maps).



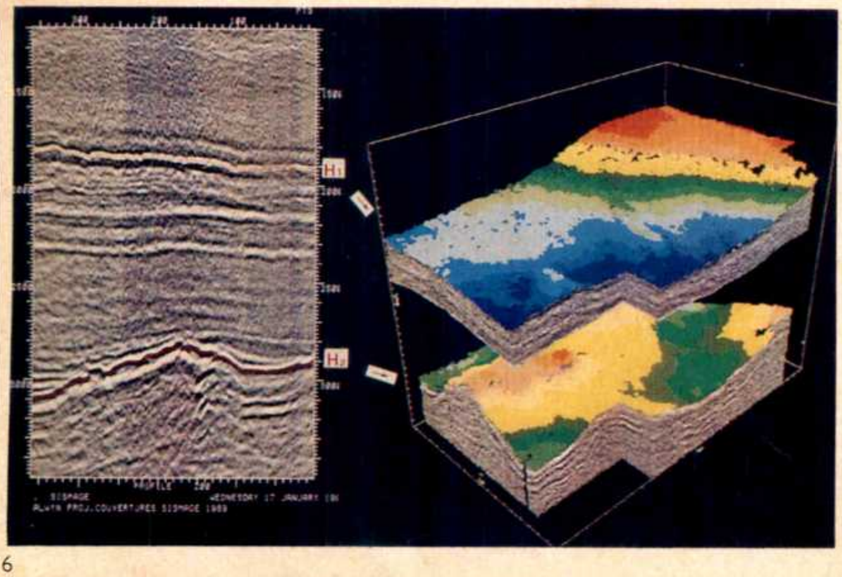
3D volume   Seismic section

Isochrons   Amplitudes

Constant time 2100twt   Interpretation

Figure 3: Amplitude maps display anomalies around faults due to acoustic attenuation effects, making them particularly useful for fault detection.

Figure 2



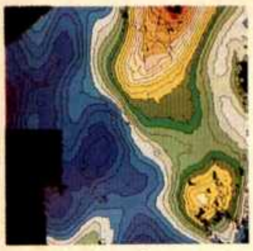


Figure 4a: Isochrons map of the Amplitude map of the North Sea Top Chalk marker

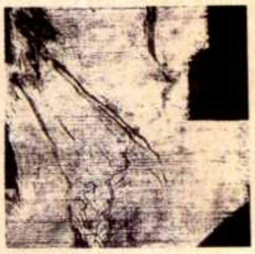


Figure 4b: North Sea Top Chalk marker

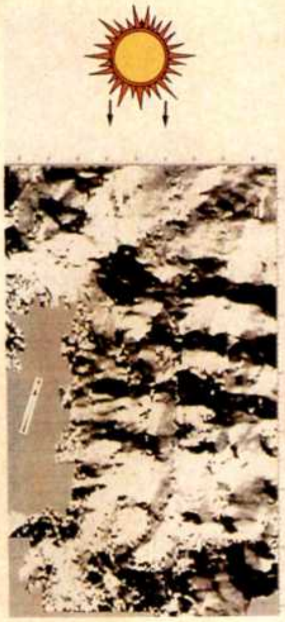


Figure 5a

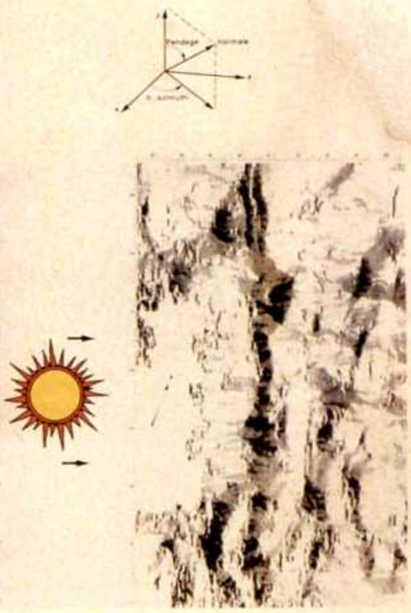


Figure 5b

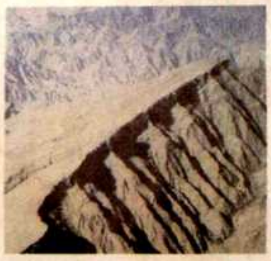


Figure 5c

Figure 5a and b: Azimuth map - the use of artificial image enhancement techniques produces an image that is very similar to an aerial photograph of similar geological features. These two figures were produced using the same map with the sun in two different positions. Figure 5c is an aerial photograph.

Amplitude maps are particulary useful for fault detection. The illustrations in Figure 3 show examples of seismic interpretation studies where the amplitude map was used to define the geological structure.

In Figures 4a and 4b the amplitude map for the North Sea Top Chalk marker demonstrates the degree of accuracy with which the structural trends are characterised. The events detected on attribute must be validated by a thorough review of the data on vertical or horizontal seismic sections.

The northern section of the amplitude map shows the complex fault pattern associated with an underlying salt dome; the events with a NW to SE shading towards the centre of the map are flexures.

Azimuth and gradient maps are now widely used for highlighting structural features. The azimuth, or direction of gradient, and the local gradient or dip, are determined by computing a plane normal to each point of a horizon. Figure 5 is an azimuth map for a recent 3D survey which shows the orientation of discontinuities independent of their magnitude.

Figure 6 shows a gradient map of horizon H1 in Figure 2. The red indicates steep gradients.

The Sismage project has also investigated the application of image processing to 2D data — which still represent about 60% of the available seismic data.

There are existing techniques for detecting major faults in 2D sections, and attempts are now being made to develop techniques for identifying minor faults, flexures and lineaments using SURFACES. The classical method for detecting faults on a 2D profile is to locate vertical shifts which consistently affect a series of seismic markers.

But the accuracy of this method is constrained by the orientation of the 2D profiles in relation to the fault plane - ideally the profile should be orthogonal with respect to the fault plane, and by the quality of the seismic markers that the fault cuts through; if they are relatively sharp the fault position can be accurately defined, if they are weak the fault position can be obscured.

REFERENCES

1) KESKES N. Surfaces new generation of 3D workstation Workshop on i.w.T EAEG 05/06/1988.

2) KESKES N.; BOULANOUAR A.; LECHEVALLIER Y.; ZACCAGNINO Ph. Image Analysis Techniques For Seismic Data. Proceeding of SEG, DALLAS October 1982.

3) KESKES N.; MERMEY P.; ZACCAGNINO Ph.:Automatic extraction of 3D seismic horizon Proceeding of SEG, LAS VEGAS October 1983.

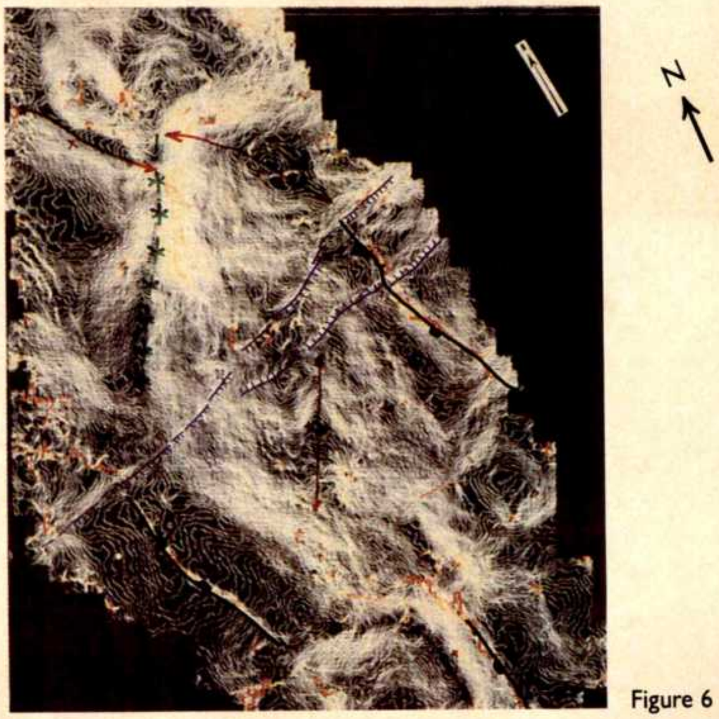
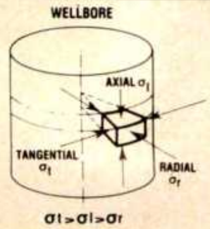


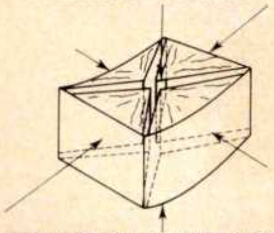
Figure 6

THE ROLE OF ROCK MECHANICS IN OIL AND GAS EXPLORATION

Vincent Maury
SNEA(P) — CSJF
26 Avenue des Lilas
64018, Pau
France



STRESSES AT THE WALL



INTERSECTION FAILURE LINES PARALLEL TO INTERMEDIATE STRESS

Figure 1

Oil and gas exploration and production is the mainstay of Elf Aquitaine's business. This article describes how advances in the study of rock mechanics are applied in the field.

In August 1987, six platforms in the Ekofisk gas field in the North Sea had to be jacked up six metres to compensate for seafloor subsidence caused by the compaction of the 3000m-deep Chalk reservoir[1]. This occurred as a direct result of hydrocarbon extraction, and is one of the most spectacular - and expensive - examples of how the behaviour of rocks can affect oil and gas production.

In recent years, rock mechanics has become an important field of study for predicting such events. Building on approaches originally developed in mining[2,30] and civil engineering[3], rock mechanics has been increasingly applied to oil exploration and production. The ISRM Congresses give an overview of work carried out since the early 1960s[4], and a symposium on Rock at Great Depths sponsored by ISRM and the Society of Petroleum Engineers (SPE), in Pau, France in 1989 reviewed all the problems encountered in oil exploration and production[5].

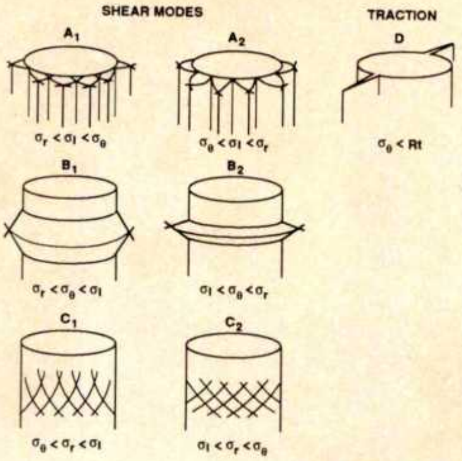
In the early 1980s the oil industry turned to rock mechanics in a bid to answer several key questions[5,6]:

1. What are the factors controlling borehole stability, and what causes borehole failure?
2. What are the effects of high temperature and pressure on wells?
3. How are the physical properties of rocks (porosity, permeability, formation factors, resistivity) influenced by depth?
4. How do in situ stresses affect hydrocarbon production, log responses and well completion procedures?
5. What happens to a field as it is depleted? Will there be gradual and widespread subsidence, will earthquakes occur, what are the expected production and environmental effects?

The prize for answering these questions is enormous - drilling offshore costs US$80,000 - $100,000 per day, and a properly positioned and engineered well is more productive than one that is not.

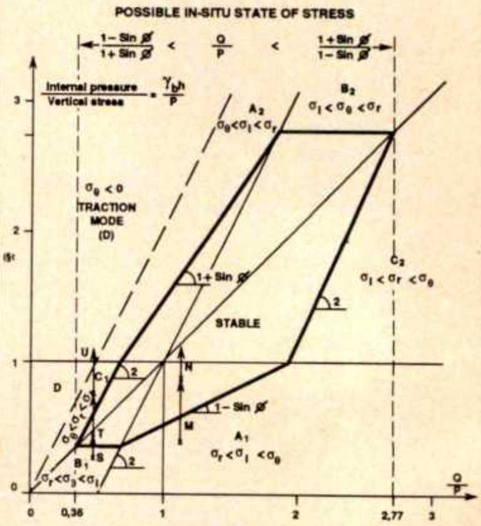
EXPLORATION PROBLEMS

Rock mechanics, including hydraulic behaviour, stress and loading history are key elements in oil exploration. Factors that must be considered include large-scale deformation (North Sea rifting) with its associated strains and failures, diagenesis, and the tectonic reactivation of


SHEAR MODES
TRACTION
A1
A2
D
$\sigma_r < \sigma_l < \sigma_\theta$
$\sigma_\theta < \sigma_l < \sigma_r$
$\sigma_\theta < Rt$
B1
B2
$\sigma_r < \sigma_\theta < \sigma_l$
$\sigma_l < \sigma_\theta < \sigma_r$
C1
C2
$\sigma_\theta < \sigma_r < \sigma_l$
$\sigma_l < \sigma_r < \sigma_\theta$

FAILURE MODES — CIRCULAR EXCAVATIONS
ISOTROPIC LATERAL FIELD

Figure 2



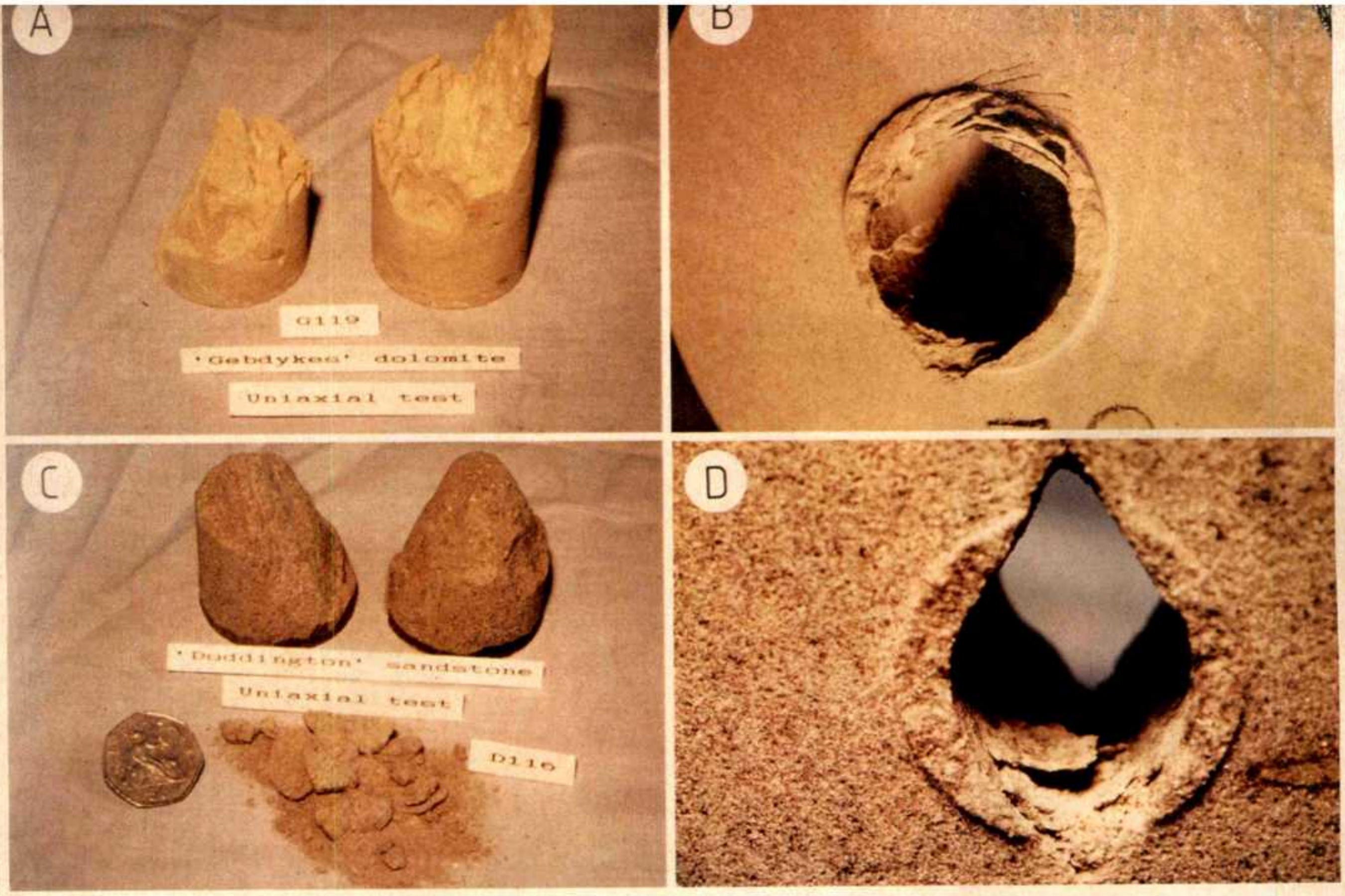
STABILITY EVALUATION (VERTICAL WELL) AND SHEAR FAILURE MODES (AFTER GUENOT)

Abscissa : $\frac{Q}{P} = \frac{\text{Horizontal stress (isotropic)}}{\text{Vertical stress}}$

Ordinate : $\frac{\gamma_b h}{P} = \frac{\text{Internal pressure } (\gamma_b \text{ mud weight})}{\text{Vertical stress}}$

Case : C = 0, ϕ = 28°

Figure 3



Oriented breakouts obtained around small scaled boreholes showing possible shear (A_1) and extension modes of failure. The effect of material behaviour on failure pattern (A and C) is clearly apparent around the hole (B and D).

basins. Associated phenomena include salt-dome formation, transformation of clays with depth and the less well understood effects of regional metamorphism. Oil and gas generation is of course dependent on the presence of appropriate organic matter, but its expulsion, migration and entrapment are influenced by thermal and rock-mechanical criteria. This is particularly true in relation to the build-up of abnormally high fluid pressures in basins[7].

Studies of various areas, including the North Sea and the Gulf of Guinea, show that reservoir pressure has a strong influence on fracturing pressure when a reservoir is depleted, and that abnormal pore pressures are always limited to 75 to 80% of the overburden stress in tectonically extensive sedimentary basins - which can be used for estimating the minimum principal stress in such basins.

The principles of rock mechanics are critical to all aspects of oil production and exploration, from large scale geomechanical surveys over tens of kilometres, to studies of the micro structure of rocks.

At the scale of tens of square kilometres, geomechanical surveys study the way targetted structures may have been formed and their relationship to potential accumulations. Hydrodynamic behaviour must be considered here[8]. For instance, are all critical faults likely to be sealed? Could fluid pressures have overcome local stresses and opened fractures so as to let hydrocarbons leak out?

At the scale of an individual oil field, production problems have to be considered. For instance, is there a system of open fractures and, if so, how did it originate? What sort of morphology characterises the fractures and how are they distributed? How will the existing in situ stresses affect drainage from these fractures during production[9]? On a smaller, centimetre scale, rock mechanics is relevant to investigations of the basic mechanisms controlling the expulsion and primary migration of hydrocarbons.

And on a microscopic scale, rock response to geophysical tools is important. For instance, although the dynamic response of a rock specimen to an acoustic wave involves stresses of the order of a millibar, the principles of rock mechanics still apply.

APPLYING ROCK MECHANICS TO THE OIL INDUSTRY

There are currently several problems in applying rock mechanics to subsurface analysis.

1. Determining the stress state at a particular point requires six measurements to record the magnitude and orientation of the three principal stresses[10]. In addition it is usually necessary to know the magnitude of the fluid pressure acting in pores and fractures. Making such composite measurements is relatively easy in mines, but much more difficult in narrow drill holes and usually it is only possible to measure one or two of the principal stress tensor components. Furthermore these procedures introduce a further stress component.
2. Current elastic or elastoplastic models do not accurately predict the mechanical response of most buried sedimentary rock. For example, small scale boreholes commonly fail at loads

REFERENCES

In the following, RAGD refers to the Proceedings of the International Symposium Rock At Great Depth (Symposium ISRM-SPE) held at Pau (France) in August 1989 (reference 5).

1) MES M.J., J. Petr. Tech. 1434-1438 (Nov. 1990)
2) OBERT L., DUVALL W. Rock mechanics and the design of structures in rock. (Wiley & Sons. New York 1967).
3) PANET M. La mécanique des roches appliquée aux ouvrages de génie civil, Ass. Am. Anc. El. Ponts et Chaussées (1976).
4) Gen. Rep. Int. Cong. ISRM Lisbon (1966), Denver (1970), Belgrade (1974), Montreux (1979), Melbourne (1983), Montréal (1987).
5) Rock at great depth, Proc. Symp. Int. ISRM-SPE, Pau (France), (1989) Maury V. & Fourmaintraux D. Ed., Balkema, Publ.
6) MAURY V. & SAUZAY J.-M., Paper 16051 SPE/IADC March 1987.
7) GRAULS D., RAGD, **3**, 1543-1550 (1989).
8) MANDL G. Mechanics of tectonic faulting, Elsevier (1988).
9) GOLAZ P., SITBON A., Delisle J., J. Petr. Techn. **42,** 8, 1032-1036 (1990).
10) CORNET F., 5th ISRM Cong. Proc. G257-286 (1983).
11) SANTARELLI F. & BROWN E.T., 6th ISRM Cong. **2** 1217-1223 (1987).
12) SANTARELLI F.J. & BROWN E.T.; Int. J. Rock Mech. Min. Sci & Geomec. Abstr. **26,** 5, 401-413 (1989).
13) MAURY V., Report of RFMUO ISRM Comm. Proc. 6th ISRM Cong. **2** 1119-1128 (1987).
14) MAURY V. Comprehensive Rock Engineering Ch. 16, J. Hudson Ed. (in press 1991).
15) GUENOT A., Proc. 6th Cong. ISRM, **1**, 109-118 (1987).
16) GUENOT A., Int. J. Rock. Min. Sc & Geom. Abs. **26,** 3/4, 185-195 (1989).
17) GUENOT A. & SANTARELLI F., RAGD **2**, 809-817 (1989).
18) GUENOT A., MAURY V. in La Thermomécanique des roches Manuel et méthodes **16** BRGM Orleans (France) (1988).
19) MAURY V. & SAUZAY J.-M. RAGD **3,** 871-883 (1989).
20) BRADLEY W.B., Oil & Gas Feb. 92-102 (1979).
21) PÉRIER P.-J., Ph.D. Univ. of California, Berkeley (1990).
22) MASTIN L. M.S. Thesis Stanford Univ. (1984).
23) PLUMB R.A., RAGD **2,** 761-768 (1989).
24) HELIOT D., ETCHECOPAR A., CHEUNG P., RAGD **3**, 1471-1486 (1989).
25) BELL J.S. & BABCOCK E.A., Bull. Can. Pet. Geol. 364-378 (1986)
26) ZOBACK M.D. & al., J. of Geoph. Res. **90,** B7, 5523-5530, June (1985).
27) KOZLOVSKY Y.A., Superdeep well of the Kola Peninsula, Springer Verlag (1984).
28) KESSELS W. RAGD **3,** 1479-1486 (1989).
29) DETOURNAY E. and CHANG A.H.D. Int. J. Rock Mech. Min. & Geom. Abstr. **25,** 3, 171-182 (1988).
30) HOEK E. & BROWN E.T., Underground excavations in rock. The Inst. of Mon. & Metall., London (1980).
31) COUSSY O. "Mécanique des milieux poreux", Ed. Technip, Paris, (1990).
32) Charlez Ph. "Rock mechanics — Theoretical fundamentals", Vol. I., Ed. Technip, Paris (1990), in press.
33) DARLEY H.C.H., GRAY G.R. "Composition and properties of drilling and completion fluids" Gulf Publishing Cy Houston (Texas, E.-U., 1988).
34) CHENEVERT M., RAGD **3** 1177-1184 (1989).
35) POLLARD D. & ATILLA A., Geol. Soc. of Am. Bull. 100, 1181-1204 (1988).
36) HUMBERT P., PIAU J.M. & SANTARELLI F.J., 7th Conf. IACMAG. (in press 1991).
37) HUMBERT P., MESTAT P. & SANTARELLI F.J., 7th Conf. IACMAG. (in press 1991).

that far exceed the rock's theoretical strength. This cannot be modelled by linear elasticity[11,12] and calls for new rheological models currently under development[36,37].

3. High temperatures, stresses and high fluid pressure in reservoirs make reliable measurements expensive and technically difficult.

However there is now a body of case histories to draw on, and some of these have provided solutions for live operational problems. Understanding stress states in many basins has in turn shed light on problems of borehole stability, as outlined below.

BOREHOLE STABILITY

To understand how a borehole behaves it is necessary to know how the rock was loaded prior to drilling, and how the near hole stresses have changed as a result of drilling. In practice not all this information is available, but it is possible to establish the following:

1. Depending on the relative values of the tangential, axial and radial stresses acting on the wall of a vertical borehole (Figure 1)[13,14], six possible shear failure modes exist (Figure 2). Tensional failure is not considered here.
2. Assuming that a Mohr-Coulomb failure criterion applies, shear failure can be inhibited by the pressure exerted by the drilling mud on the borehole wall. The pressure required to maintain borehole stability will depend on the relative magnitudes of the lateral stresses, Q (Figure 3), after Guenot[15,16], here assumed isotropic.

   In a regime with high horizontal isotropic stresses $Q > P$ in Figure 3, borehole stability is also a function of the mud weight (M to N). If the horizontal stresses are low ($Q < P$, in Figure 3), hole stability is also a function of mud weight, but here the mud weight range is much narrower (S to T), before hydraulic fracturing is initiated by tensional failure at the borehole wall (T to U).
3. If the stress field is anisotropic horizontally ($Q_1 > Q_2$)[14], more complicated modes of failure may be involved. Usually the greatest stress concentration will occur along the diameter where the smaller horizontal stress intersects the borehole wall and will lead to oriented breakouts (anisotropic mode A_1 in Figure 2).
4. The above analysis can be used for an overbalanced situation where the drilling fluid can percolate into the formation, or for underbalanced drilling where mud pressure is less than formation pressure[16]. The same type of analysis can be made for inclined or horizontal wells.
5. Thermal effects also influence borehole stability[17,18]. The drilling fluid is warmed by passing through the bit nozzles, but it is still significantly cooler than the surrounding rocks at depths greater than 1500-2000 metres. Thus the borehole walls are cooled by $\delta\theta$ which induces tensile stresses $\delta\sigma$ that alleviate the acting compressional, axial and tangential stresses by $\delta\sigma - E\alpha \times \delta\theta/(1-\mu)$ where E, μ, α are respectively, the elasticity modulus, Poisson's ratio and the coefficient of linear expansion of the rock. On the other hand, in the upper part of a drill hole, the surrounding rock is heated by the drilling mud. This heating increases the compressional stress concentration induced by drilling the hole, increasing the tendency for the walls to cave in[17].
6. Drilling mud engineers aim to minimise fluid penetration into the formation by formulating drilling fluids that deposit a mudcake on the walls of the borehole. In practice this mudcake acts as a membrane and exerts pressure on the wall. Drilling mud can also penetrate into faults, fractures and joints, raising fluid pressure in these openings and reducing pressure on the opposing joint surfaces. This can induce local movements large enough to shear boreholes or even completed wells[19].

These principles have led to fundamental advances in the understanding of problem wells and have helped reduce operational drilling problems. Recent laboratory studies[21,22] have produced observations of failure in accordance with theoretical predictions. New logging tools like BHTV and FMS provide images of borehole failure[23,24]. Material from failed boreholes is carried to the surface in the returning drilling mud, often providing information on the type of failure that is occurring.

After drilling a few wells in a basin, an accurate evaluation of the in situ horizontal stresses can be made, providing information on the stability of subsequent wells and the preferential drainage of reservoirs - and thus helping in the planning of an exploration or development of a field.

Systematic analysis of borehole breakouts[25] becomes a powerful tool for mapping the principal stress directions, which in turn allows the evaluation of the direction and magnitude of current tectonic thrusts. These studies will be very useful in the Russian Kola[27] and the German KTB ultradeep well projects[28].

FUTURE PROSPECTS

Research is now being carried out in the following areas:

- The isothermal[29] and non-isothermal mechanical behaviour of saturated porous media. The thermodynamic approach by Coussy[31,32] analyses these problems in relation to thermal, hydraulic and mechanical effects.
- The behaviour of shales[33,34] — these very abundant rocks react to drilling fluids and may swell to the point of completely destabilising a well.
- Understanding rock fracturing at a geological scale — the origin of certain joint sets is still unknown [35] and while current research in bifurcation theory is promising, it has yet to differentiate between what is due to material behaviour, and what to loading history.

Structures in colloidal physical chemistry

J. Prost & F. Rondelez

The vast range of technological and industrial applications of colloidal systems is largely a direct result of their diversity of structure on microscopic and mesoscopic scales. Recent progress in understanding these structures allows for greater control of the macroscopic properties, as well as providing new avenues of fundamental research in physics, chemistry and materials science.

COLLOIDS have been defined classically as systems involving characteristic length scales ranging from 10 to 1,000 nm (we will also call such systems 'mesoscopic'). Such a definition based on a quantitative measure contrasts with most modern ones, which are based on qualitative criteria such as the system's symmetry properties. This definition has, however, the pragmatic value that it immediately implies the ubiquity of colloidal systems in the natural world. They include, for example, suspensions of particles, one-component and multicomponent systems, gels, rubbers, foams, emulsions and paints. Despite their diversity, all of these systems share the feature of exhibiting strong responses to modest external perturbations. We will show here how this behaviour results from the existence of certain types of ordering at length scales much larger than atomic, even amidst apparently complete macroscopic disorder.

The study of colloids as a well defined field of research has been active ever since Graham first made the distinction, in 1861, between colloidal and molecular solutions[1]. Modern concepts, such as connectivity transitions and the interplay between short- and long-range order, are now employed to understand the macroscopic properties of these systems. Although much of the technological interest in colloids results from their dynamic (rheological) behaviour, we focus here largely on static structures, the characterization of which is a prerequisite to a more general understanding of these systems.

Classical systems

Graham[1] classified a suspension of particles of silver bromide in water as colloidal because the particles cannot diffuse through a membrane with a pore size smaller than 10 nm. This contrasts with sodium chloride solutions, which can flow through the same membrane without change in composition. The upper limit in 'particle' size for a colloidal suspension is defined by the point at which brownian motion, which tends to maintain the solution in a dispersed state, is superseded by the gravitational force which tends to segregate and settle the particles from solution. The lower limit in size is less well defined: the particle size must be large enough that a continuum description of the system is still applicable. Van der Waals and electrostatic forces are generally the source of attractive or repulsive physical interactions between the suspended particles. On the other hand, continuum hydrodynamic theory was used by Einstein[2] to discuss the viscosity of colloidal suspensions as a function of the particle density.

Latex particles and soap micelles have become two of the most studied examples of colloidal systems. The former are heavily cross-linked polymer particles which behave as rigid balls[3]. They can be prepared over a large range of diameters, typically from 20 nm up to several micrometres, with a remarkably small polydispersity (size distribution). The latter are formed from amphiphilic molecules such as long-chain fatty acids or phospholipids. In water, these tend to gather into spherical aggregates (micelles) in which the polar heads face outwards, encountering the polar water molecules, and the hydrophobic chains lie buried in the aggregate's core[4]. The micelle's diameter is controlled by the chain length. For the common industrial detergent sodium dodecyl sulphate, each micelle contains about 60 molecules and has a typical radius of 1.8 nm. For obvious reasons, these colloids are called self-assembling and exhibit reversible behaviour. They redissolve into their constituent molecules if the soap concentration is lowered below a specific value, called the critical micellar concentration. Micelle dissolution can be avoided, however, by cross-linking if the molecules bear a polymerizable group such as a carbon–carbon triple bond.

The Deryaguin–Landau–Verwey–Overbeek (DLVO) theory[5] provides the classical description of these suspended particles, based on a consideration of the two principal forces involved (electrostatic and van der Waals). The van der Waals interaction is always present: it is due to the difference in polarizability between the particles and the solvent. For identical particles, the interaction is always attractive and, for $d \gg R$ (where R is the particle radius and d is the interparticle distance), it scales as H/d^6. Here H is the Hamaker constant, which is related to the square of the polarizability mismatch. When the molecules bear ionizable groups, there is also an electrostatic repulsion, which is screened at long distances by the counter-ions that are always present in the solution (this screening can be enhanced by addition of a salt). The electrostatic energy is described by a screened Coulomb potential $\exp(-d/\lambda)/d$, where λ is the Debye–Hückel screening length.

The combination of these two opposing forces has been generally found sufficient to describe the stability of colloidal suspensions. If electrostatic repulsion is large enough, the solution is stable against aggregation. Van der Waals attraction can always be made dominant, however, by adding a sufficient amount of salt. This is the reason why estuarine waters are muddy: the clay particles transported by the river coagulate when they encounter the saline waters of the ocean. Conversely, a weakly stable non-aggregated system can be made more resistant to flocculation by adsorbing long-chain polymers onto the particles. Polymer chains do not tend to interpenetrate for entropic reasons, so they form a protective layer which prevents particles from approaching within a distance smaller than twice their characteristic size. This reduces the van der Waals attractive energy to a value smaller than the thermal energy kT, thus stabilizing the suspension. This kind of polymer stabilization was known to the Egyptians as early as 2000 BC. Indian inks are colloidal suspensions of carbon-black particles in water stabilized by the addition of natural gum.

Colloidal suspensions have been used to study crystallization in 'ideal' systems in which colloidal particles represent atoms. Because all distances are thereby scaled up, the structures become much easier to visualize[6], and the electron microscope of the metallurgist can be conveniently replaced by an optical microscope. Moreover, two-dimensional systems can be studied as well as three-dimensional ones. Layers of polystyrene balls of micrometre size suspended at the air–water interface have been widely used to study two-dimensional crystallization[7]. Alternatively, such a colloidal suspension can be confined between two optically transparent plates spaced apart by no more than two or three particle diameters[8].

One major difficulty exists in this approach, which has been overcome only recently by careful sample preparation. As the interaction forces are generally a strong function of the particle size, any dispersion in this size automatically leads to crystalline disorder and to the formation of a glass rather than of a crystal. When polydispersity is suppressed or markedly reduced, crystals are readily obtained[9].

Three principal types of monodisperse systems have been described: (1) latex particles of crossed-linked polystyrene or polymethyl methacrylate stabilized by ionizable surface groups in water[3]; (2) polymer particles stabilized sterically by surfactants[10]; (3) oil-in-water emulsions of silicone droplets which have been rendered monodisperse by careful fractionated crystallization[11]. In all these cases, particle sizes range from a few micrometres down to a few thousand ångströms.

Polystyrene latices have been used most commonly. They carry charges as high as 1,000 e (e being the charge of an electron) and so they behave as macro-charges interacting via a screened Coulomb potential. These systems can be characterized by three lengths: the particle diameter ϕ, their average separation d, and the Debye screening length λ. For $d/\lambda \gg 1$ and $d/\phi \approx 1$, they behave as a collection of hard spheres with a radius equal to the screening length. For $d/\phi \gg 1$ and $d/\lambda \ll 1$, they are equivalent to a classical one-component plasma in a uniform background of neutralizing charges[12]. Hard spheres crystallize in close-packed structures (hexagonal compact or face-centred cubic) whereas a one-component plasma crystallizes in a body-centred cubic array. Both types of behaviour have been observed experimentally by changing the ionic strength of the solution (that is, changing λ).

Localized defects in the crystalline structure can be introduced by mixing particles of different sizes. Above a critical concentration of defects, the crystal either melts or becomes a glass, depending on the volume fraction[9]. Dislocations and grain boundaries in crystals have been seen optically.

Charged latex particles do not mimic atoms very accurately because they interact mainly through repulsive forces. Polymethyl methacrylate particles suspended in water at low pH, coated with poly-12-hydroxy stearic acid, attract each other at long distances according to the $1/d^6$ (non-retarded) van der Waals interaction, and repel each other sterically at short distances because of entropic interactions of the stearic acid coats. Thus they provide a mesoscopic analogue of spherical atoms interacting via hard-sphere potentials. Such suspensions have been shown[13] to exhibit a liquid–solid coexistence region for particle volume fractions between 0.494 and 0.545. Crystallite sizes in these colloidal suspensions are generally in the range 10–50 μm, which is sufficient to perform optical diffraction. Powder diffraction patterns show that the packing is predominantly face-centred cubic, as expected for hard-core repulsions[14]. But the energy difference between this and other ordered structures seems to be extremely small, and this may explain why, even in the case of slow growth, the crystals still contain many stacking faults.

Classical DLVO theory is not always sufficient to predict the macroscopic behaviour of colloidal solutions, especially in multicomponent systems. For instance, when free polymer chains are progressively added to a solution, colloidal stability can be decreased markedly above a certain polymer concentration[15]. Similarly, when short-chain surfactants are added to a suspension of solid colloidal particles, strong attractive forces can appear above a certain concentration of surfactants, leading to separation of a dense colloidal phase from a more dilute one.

In both of these cases, the colloidal destabilization is due to a subtle 'depletion' interaction. Suppose that the added component (the polymer chains or the short-chain surfactant) tends to become adsorbed on the particles. It will do so only as long as the particle surfaces are not saturated. After saturation is reached, the additional polymer chains remain free-floating objects with a typical coil size R_g. In the case of surfactant molecules, they clump into micelles of size R. These objects are not able to penetrate the space between two colloidal particles if these particles are separated by a distance less than R_g (or R)[16]. This leads to inhomogeneous concentrations within the solution and therefore to differences in osmotic pressure. As the osmotic pressure is less in the depleted zones between the colloidal particles, two neighbouring particles are pushed closer together. This attractive force tends to aggregate the colloidal particles into flocs if there is not a strong repulsive barrier against adhesion. If the only repulsion is hard-sphere-like, the particles form close-packed structures. This behaviour is observed for emulsions of silicone oil in water stabilized by sodium dodecyl sulphate molecules[11]. These surfactants are adsorbed at the oil–water interface, producing kinematic stabilization of the emulsion by reducing the van der Waals attraction between the oil droplets. At low surfactant concentration, the emulsion is stable for years. As the surfactant concentration is increased above roughly the critical micellar concentration, phase separation takes place between an oil-rich phase and a water-rich phase. The former is composed of a close-packed array of silicone-oil droplets, in which long-range crystalline order is revealed by sharp Bragg diffraction peaks at visible wavelengths. The structure is face-centred cubic, as expected for an array of hard spheres. The adsorbed surfactant molecules thus create a high energy barrier against droplet coalescence at short range, but simultaneously the surfactant micelles provide the attractive force necessary for condensation.

Polymers can also provide attractive forces between micelles of surfactants by a 'bridging' mechanism. Under certain conditions, a single isolated macromolecule can be adsorbed simultaneously on several micelles. This is particularly likely when adsorption forces are weak or when the micelle size is very small compared with the dimension of the macromolecule. The polymer–micelle complex that forms resembles a necklace in which micelles are 'threaded' on the polymer backbone. In a mixture of polyoxyethylene and sodium dodecyl sulphate in saline water (0.4 M sodium bromide), a single polymer with 3,000 monomers becomes attached to about 30 micelles, with about 60 monomers effectively bound to each sodium dodecyl sulphate micelle[17]. Unbound polymer strands connect the adsorbed chain segments to each other. The fraction of non-adsorbed to adsorbed segments is determined by a balance between the energy gained in the adsorption process and that lost because of the stretching of the polymer segments owing to the Coulomb repulsion between the micelles. The amount of stretching can be controlled by changing the ionic strength of the solution. Sodium dodecyl sulphate micelles can also be interconnected by denatured proteins such as bovine serum albumin or phosphorylase[18].

Industrial colloids

Having described the use of polymer chains for stabilizing and destabilizing colloidal solutions of solid particles or of emulsions, we now discuss systems in which permanent or temporary random networks of aggregates control the system's properties. The aggregates may be cross-linked polymers or gels. The latter may be obtained, for example, by flocculation of colloidal silica suspensions as in ceramic processing techniques, or by flocculation of oil-in-water emulsions as used in cheese processing. In these two examples, colloidal suspensions of, respectively, silica spheres or oil droplets coated with a protein (casein) are stabilized electrostatically as described earlier, and the addition of an appropriate salt triggers aggregation, leading to the formation of coagulated networks which can percolate throughout the available space—in other words, to the formation of a gel. Many systems may be described in this way; here we illustrate some, such as bitumen and greases, that are of particular importance to the oil industry. Connectivity between surfaces, rather than particles, can also be achieved, as in the case of foams, for example.

Polymers and gels. It is hard to imagine something more disorganized than long, flexible macromolecular chains dissolved in an aqueous or organic solvent. Each polymeric chain is composed of thousands of identical monomers, linked at either end but with rotational degrees of freedom along the backbone which make them highly flexible[19]. Moreover, the local conformation of the chain is constantly changing with time because of brownian motion. The only restriction of these conformations is dictated by the fact that two monomers cannot occupy the same space at the same time (the excluded-volume effect).

The best measure of the size of such a polymer is its time-averaged end-to-end distance, or equivalently its radius of gyration R_G. If N is the number of monomers in a single chain and a is the monomer length, then $R_G \sim aN^\nu$, where the exponent ν can take three universal values depending on the thermodynamic 'quality' of the solvent[20]. For 'poor' solvents, each monomer is preferentially surrounded by other monomers rather than by solvent molecules: the chain thus collapses into a dense globule containing almost pure polymer. In this case, $\nu = 1/3$. Very important in practice are the alternative values $\nu = 1/2$ and $\nu = 3/5$ for ideal and swollen chains respectively. Ideal chains correspond to the case in which the effective two-body excluded-volume interaction vanishes. The temperature at which this occurs is called the theta point. Swollen chains correspond to the case in which repulsive excluded-volume interactions are dominant over attractive van der Waals interactions. Such a situation is generally encountered for temperatures several tens of degrees above the theta temperature[21].

Each chain in solution occupies a volume R_G^3 that is considerably larger than the actual chain volume: the volume pervaded by a single chain is about 99% solvent and 1% polymer. This has the important consequence that as more chains are added to the solution, they are able to overlap to form a complex random network of entangled chains. In this situation, the characteristic length in the problem is no longer R_G but a smaller distance ξ, which is proportional to the average distance between two entanglements[20]. ξ decreases as the polymer concentration increases, being of the order of R_G at the concentration at which overlap first occurs and of the order of a in the pure polymer melt.

These polymer solutions can be viewed as a network with a mesh size ξ. This network can be defined only statistically and is constantly changing its conformation. Nevertheless, the motion of solid particles thrrough the network depends strongly on their size R relative to ξ (ref. 22): for $R > \xi$, the motion is hindered by the polymer network, whereas if $R \ll \xi$ the object does not see the chains and moves as if in a pure solvent. This provides the basic mechanism for gel-permeation chromatography.

In many applications it is preferable to form permanent rather than temporary cross-links. This is possible if each polymeric chain terminates in a chemically active group capable of forming covalent bonds with cross-linking agents. The latter are generally low-molecular-weight compounds containing several functional groups that can attach simultaneously to several polymer chains. A classical example is that of chains terminating in acid groups, which can be cross-linked by trialcohols via a condensation reaction. The gel that is thus formed consists of a polymeric cluster of essentially infinite molecular weight trapping a large amount of solvent[23]. The characteristic length of the permanent network is the average distance ξ' between two cross-links. This distance depends not only on the molecular weight of the individual chains between two cross-links but also on the solvent fraction. There is, however, an optimum swelling ratio, as a result of the competition between two effects: the osmotic pressure of the solvent, which tends to dilute the chains and therefore to increase ξ', and the elastic response of the chains to stretching.

An ideal dry gel brought into contact with an infinite reservoir of solvent will swell until ξ' becomes of the order of R_G, the radius of gyration of the chains between two cross-links; but for subtle reasons, it is difficult to test this experimentally[24]. On the other hand, the effect of swelling on the gel's shear modulus and cooperative diffusion coefficient have been well studied[25,26]. The swelling is particularly pronounced when the chains carry dissociable ionic groups and the solvent is water. Because of the electrostatic repulsion between the polar groups, the chain backbone adopts a rod-like conformation with a radius of gyration $R_G \sim N$, which is considerably larger than the corresponding value $R_G \sim N^{1/2}$ or $R_G \sim N^{3/5}$ for a neutral polymer of identical molecular weight[27]. Polyelectrolyte gels can absorb 1,000 times their dry weight[28], a property that has been put to use in highly absorbent diapers or for the slow release of water to house plants.

So far we have discussed only gels formed by covalent bonding. Many natural gels are binary solutions that acquire remarkable mechanical properties through a reversible aggregation process that does not involve such bonding, but is controlled instead by conformational changes. A typical example is gelatin, the denatured form of collagen. Collagen is the most common protein in mammalian tissue, being a component of tendons, skin, bones and cornea. It is a rod-like protein made of three strands wrapped together in a triple right-handed helix with a pitch of 9 nm, each strand being a left-handed helix of much smaller pitch (0.9 nm). The structure is stabilized by hydrogen bonding between the CO and NH groups in the peptide linkages of adjacent chains; it is therefore very sensitive to temperature[29], and at sufficiently high temperatures collagen will denature into the three separate strands. Each isolated strand has the random-coil conformation typical of flexible long-chain macromolecules in solution. An aqueous solution of collagen heated above 50 °C therefore behaves as a typical low-viscosity sol. On cooling, monomers of different chains tend to regroup themselves into progressively longer helical sections, and may eventually restore the original native collagen structure. But as the process occurs at random, any given chain connects with several others, thus forming a three-dimensional network with the helical sections serving as the cross-links (Fig. 1). The behaviour of the gelatin system has been analysed within the framework of percolation theory[30]. It has been found that the helix fraction is the relevant parameter that controls the transition from the sol to the gel phase. Above a critical helix fraction f_c, the elasticity modulus

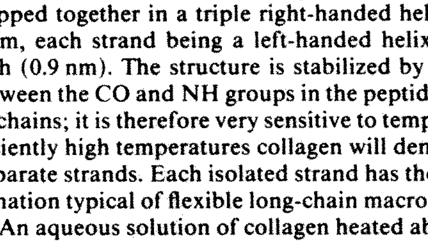


FIG. 1 Schematic representation of the reversible aggregation process of gelatin, leading to physical gelation.

E increases according to a power law $E \sim (f - f_c)^t$, where t is a characteristic exponent equal to 2 ± 0.1. Conversely, below f_c newtonian behaviour is observed (viscosity independent of flow velocity) and the shear elastic modulus is zero.

Recently, an interesting connection was established between linear, flexible polymer chains and micelles of short-chain surfactants. Under certain conditions, determined for example by the nature of the amphiphilic molecules, the presence and the nature of added salt, the temperature and so on, the small spherical micelles that are generally formed at low concentrations undergo uniaxial growth, transforming into long, flexible micelles which behave as if they are themselves polymer chains when the energy required to form an 'end cap' is large enough[31]. As is the case for regular polymers, these elongated micelles become entangled even at low concentrations, and it is possible to define an average distance ξ between entanglements which does not depend on the size of the individual chains but only on their overall volume fraction. The main difference with classical polymers is that these elongated micelles can break and reform continuously[32]. The width of the molecular-weight distribution is therefore controlled by thermal equilibrium and consequently cannot be made very narrow. For this reason, these elongated micelles are often called 'living' or 'equilibrium' polymers. In the entangled configuration, they can raise the viscosity of the solution by several orders of magnitude.

Once no more than a curiosity, living polymers have now been observed in several classical colloidal solutions, both aqueous and non-aqueous, such as cetyltrimethylammonium bromide or cetylpyridinium bromide in the presence of potassium bromide[32], and lecithin plus a trace amount of water in isooctane[33]. Despite little knowledge of their structure, they have been used industrially for some time as a thickening agent in shampoos.

Greases. The macroscopic behaviour of lubricating greases is similar to that of rigid gels. Microscopically, however, they are quite distinct, being formed from short-chain surfactants, such as lithium 12-hydroxy stearate, dispersed in a mineral oil. The surfactant molecules aggregate into fibres which provide the long macromolecular strands necessary for gel formation[34]. Small-angle neutron and X-ray scattering show that these fibres consist of ribbons of a lamellar phase, with a finite lateral extent ~10 nm, and a thickness ~5 nm, corresponding to a bilayer with the polar head groups on the inside and the aliphatic chains on the outside[35]. Early work on dry soaps showed that such ribbons result from a combination of amphiphilic interactions and packing considerations[36]. Electron microscopy reveals (Fig. 2) that the fibres are twisted around the long axis. This is consistent with the fact that 12-hydroxy stearic acid possesses an asymmetric carbon atom, and explains why the grease exhibits strong circular dichroism and optical rotation.

Electron microscopy of the dried gel suggests that the contact points that are necessary to stabilize the network result from the random twisting together of independent fibres over relatively large distances, in a manner similar to the formation of interwoven collagen triple helices in gelatin at low temperature. In lubricating greases, the resistance to external shear stress is controlled by varying the length and the number density of these contact points.

Greases are thus a striking example of a complex self-assembling system. The lamellar arrangement maximizes the van der Waals interactions between the soap molecules and screens their polar heads from the non-polar mineral oil. As we show later, an alternative to the formation of twisted fibres is the clustering of ribbons into a hollow cylindrical shape.

Other structures are possible for associations of amphiphiles in a non-polar matrix. For instance, one can attach a few ionizable groups onto a polymer chain backbone, either at selected positions (such as the extremities) or at random. As such groups are immiscible in a non-polar solvent, they form clusters, cross-linking the polymers and again resulting in the formation of a three-dimensional network. The associative forces in this case are, however, weak and can easily be broken by the application of an external mechanical stress. Such polymers thus exhibit reversible shear-thinning behaviour, which is useful, for example, in the development of non-drip paints and drag-reducing agents. When conventional polymers are used for this latter purpose, most will undergo chain scission by breaking of covalent bonds at high shear stresses and are thus degraded irreversibly. For polymers associating via polar clusters, however, the network is broken only temporarily when stress is applied, reforming as soon as the applied stress falls below a certain threshold[37]. In these systems, the gel structure exists in the static state but is destroyed when shear is applied; the reverse is also possible if the clusters are built preferentially from polar groups attached to the same chain. In the quiescent state the chains are collapsed because of this internal cross-linking, but a shear stress breaks up the internal clusters, allowing the chains to expand so that inter-chain cross-linking is possible to form a highly viscous gel-like structure[38]. These shear-thickening colloidal fluids may serve as vibration dampers.

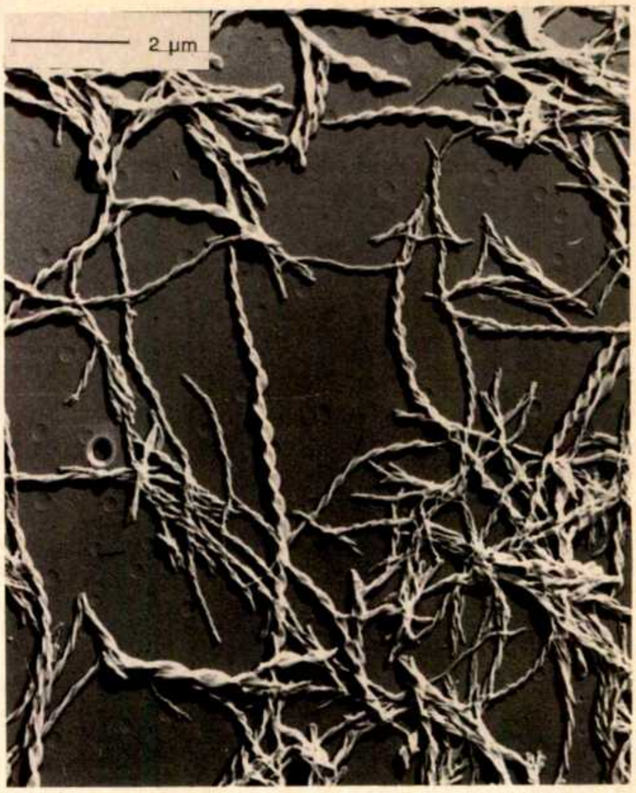


FIG. 2 Electron micrograph of a typical lithium stearate grease. The threads are crystals of lithium stearate, two layers thick and a few hundred ångtröms wide, agglomerated in bundles (magnification ×15,000). (Courtesy J. M. Thiebaux, Elf Aquitaine.)

Bitumen. Bitumen seems to be a highly unattractive system to study. This dark-brown by-product of petroleum distillation is an exceedingly complex mixture of more than one thousand different types of hydrocarbon: linear or cyclic, paraffinic, aromatic or naphthenic, saturated or unsaturated, with or without functional groups or heteroatoms. The molecular weights of these various constituents can be anywhere between 600 and 2,000 daltons. Moreover, bitumen contains large molecular aggregates called asphaltenes. Bitumen is therefore a colloidal suspension[39], in which the asphaltenes are the solute particles (with an average size of 100 nm) and the saturated paraffins, aromatic oils and resins constitute the solvent.

The macroscopic properties of this suspension are largely dependent on the volume fraction of asphaltenes[40]. At low volume fractions, the viscosity is newtonian and increases linearly with the volume fraction of solute particles. At volume fractions higher than about 8%, however, the rheological behaviour changes drastically: bitumen becomes a viscoelastic gel with a non-zero elastic modulus, indicating that a three-dimensional network of asphaltene particles has been formed. Unlike ordinary fluids, the network is able to sustain static shear. The asphaltenes interact by relatively weak van der Waals forces, which is why bitumen with 15% asphaltene content, solid at room temperature, becomes a fluid above about 60-100 °C.

Bitumen is a thermoplastic material, and many of its properties have long been known and exploited. Romans sent slaves to collect it from the shallow beds of the Dead Sea, and would use it as a watertight sealant in triremes. It was also used by the Babylonians as mortar in building construction. The recent elucidation of its underlying physical structure and thus a better understanding of its properties has now made possible the development of new, more effective materials. The gel structure of bitumen can be made more rigid, for example, by incorporating an artificial polymer into the structure. Typically one uses a triblock copolymer A-B-A composed of two sequences A that are immiscible in the bitumen and one sequence B that is miscible. The short A sequences agglomerate into nodules, in much the same way as polar head groups of amphiphiles pack into reverse micelles in a water-in-oil emulsion (Fig. 3). These nodules act as cross-links while the long B chains provide the flexible spacers between them. It is also possible to use diblock A-B polymers with a small amount of cross-linking agent to couple the B chains together at a few points, forming an equivalent of the triblock polymer *in situ*. This latter approach facilitates the incorporation of the high-molecular-weight B chains into the bitumen, and has the additional advantage that cross-linking takes place simultaneously between the asphaltenes and the B chains. One is thus left with two interpenetrating networks, one formed by the native asphaltenes and the other by the added polymer chains. High-grade bitumens formed in this way are used in road construction and roof waterproofing.

Self-supporting soap films and foams. Self-supporting soap films are a special case of foams, consisting of a thin liquid layer separating two (essentially infinite) volumes of gas. A freshly formed soap film has a thickness of a few hundreds of nanometres and is comprised of a water layer sandwiched between two monolayers of surfactant molecules[41]. The polar head groups of the amphiphiles face the water phase and the aliphatic, hydrophobic hydrocarbon tails are directed towards the vapour phase. Once formed, a soap film attached to a glass frame held vertically starts to drain by gravity and by the suction produced by the pressure gradients that exist between regions of different curvature. This thinning can be monitored by observing the film in reflection, under white-light illumination: the colour depends on the phase difference between light rays reflected from the front and the back layers of the film and therefore is a function of film thickness. The colour is black for film thicknesses in the range 5-30 nm, and silvery white for thicknesses from 30 to 120 nm. For greater thicknesses up to $\sim$370 nm, the colours range through the visible spectrum. With the exception of magenta (at $\sim$201 nm thickness), these colours correspond to film thicknesses equal to twice the respective wavelengths of light (second-order interference). If the film does not rupture during thinning, it will eventually acquire a uniform hue, generally black or silver-white, indicating that the film has reached its equilibrium thickness. This equilibrium value is determined by the competition between two kinds of interactions: attractive van der Waals forces acting between the two surfactant monolayers through the water layer, and repulsive electrostatic forces between the ionized polar head groups. (In the case of non-ionic soaps, the long-range electrostatic Coulomb repulsion is replaced by a shorter-range repulsion between the hydration shells of the hydrophilic heads[5]. In general, the interaction potential resulting from the addition of these two opposing forces exhibits two distinct minima. There are therefore two possible equilibrium thicknesses for the film. The largest corresponds to the so-called common black film and is in range 10-100 nm, whereas the smallest corresponds to the Newton black film and is of the order of 5 nm (ref. 42). A draining soap film will generally become a common black film. If the energy barrier between the two equilibrium states is comparable to kT, however, which may be arranged by adding electrolytes to the aqueous solution to screen the electrostatic interaction, a common black film will transform to a Newton black film very readily (for example, as a result of an external mechanical disturbance).

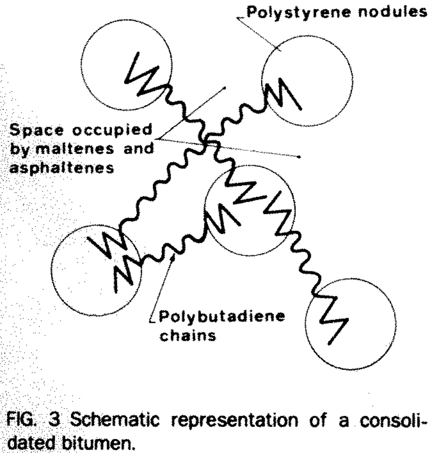


FIG. 3 Schematic representation of a consolidated bitumen.

Blowing a stream of air onto a self-supporting film may cause it to detach from the frame and close on itself to form a spherical bubble. A foam is an array of bubbles—a mixture of liquid and gas stabilized by surfactants[43]. Foams are thus related to emulsions, in which the dispersed phase is another liquid rather than a gas.

If the foam contains large amounts of liquid, the gas bubbles are widely separated from each other and remain spherical. The gas pressure P_1 inside a bubble of radius R is given by the Laplace law $P_1 - P_0 = 2\gamma/R$, where P_0 is the pressure in the liquid phase and γ is the liquid-gas interfacial tension. Thus small bubbles have higher internal gas pressure than large bubbles. If the foam contains a distribution of bubble sizes, the larger ones will therefore grow and the smaller ones shrink by gas diffusion through the liquid phase. This disproportionation[44] is equivalent to the evaporation-condensation mechanism of Ostwald ripening long known to occur in two-phase separation of binary alloys[45].

If the volume fraction of liquid falls below 5-10%, the foam is called 'dry' and consists of polyhedral gas bubbles separated by thin films of liquid. Because the pressures inside contiguous cells are nearly equal, there is no significant gas diffusion between the cells. The foam's evolution is governed by liquid drainage under gravity and by capillary suction forces. The liquid films between adjacent cells gradually thin out at a rate controlled by the fluid viscosity and specific gravity. As the film thickness decreases below 100 nm, additional long-range molecular forces, such as the van der Waals attraction between the two sides of the soap film, become important, and the thinning rate increases sharply. If the equilibrium thickness is of the order of 20 nm, this process leads to sudden 'black spot' formation: the foam becomes extremely fragile and can rupture spontaneously under the influence of thermally driven capillary waves, which creates holes in the film.

The geometrical properties of three-dimensional polyhedral foams were studied by Plateau as early as 1869[46], who summarized them as follows:

(1) Along one edge, three (and only three) liquid lamellae meet. (2) At an edge, liquid lamellae are equally inclined to one another and hence their dihedral angle of inclination is 120°. (3) At one vertex, four (and only four) edges meet, and the angle at which they meet is the tetrahedral angle 109° 28′.

The topology of these interconnected polygons is still a subject of intense interest[47]. One reason for this interest is that they represent an example of the mathematical problem of finding the surfaces of minimal area contained between fixed boundaries. This is because the free energy of the soap film, which thermodynamics tends to minimize, is proportional to its area. This explains why soap froths are not fragile despite their extremely low density: any deformation of the structure displaces the system from its minimum surface energy and generates a strong elastic restoring force.

The combination of low density and reasonably good mechanical resistance is useful for applications of foams in fire-fighting[47]. Gasoline fires can be extinguished by covering the flames with a thick, semi-rigid foam blanket which minimizes the contact with air. The low density allows the water in the foam to float even though it is generally denser than the burning oils. An aqueous foam with bubbles about 1 cm in diameter separated by films of about 10 μm in thickness has a density of only 3×10^{-3} g cm^{-3}. The chemical composition of the foam can also be varied to optimize its fire-fighting utility. For instance, it has been found empirically that fluorocarbon surfactants are less soluble than hydrocarbons in gasoline, which always contains traces of polar organic liquids such as alcohols and ketones. They are also much more heat resistant. In combination, these considerations make even small volumes of fluorocarbon foam effective in extinguishing fires.

Another characteristic of foams that can be put to industrial use is that they have extremely arge specific areas. This property is used in ore extraction by the flotation method. Under certain conditions, the ore powder is absorbed at the air–water interface and stays suspended in the foam by capillary forces, while the more-compact rocks and pebbles sink in the liquid. The amount of ore that can be separated for a given amount of liquid is directly proportional to the surface area of the foam; a foam with a specific area of 0.2 m^2 g^{-1} can easily separate a mass of ore a thousand times larger than its own mass of liquid.

It is sometimes desirable to decrease artificially the stability of soap films. This is generally done by adding to the solution solid particles which become trapped in the fluid film and promote its rupture. One of two different mechanisms is generally involved, depending on the particle size R relative to the film thickness δ (ref. 47). If $R>\delta$, the particle protrudes on both sides of the film. If, in addition, the particle is hydrophobic, there will be a finite contact angle between the fluid layer and the surface of the solid particle. This local film curvature creates an excess Laplace pressure which causes the liquid to flow away from the particle and therefore to thin out. The activation energy to create a hole is of the order of $\gamma\delta^2$, where γ is the air–liquid interfacial tension. It therefore rapidly decreases as δ decreases, being of the order of 10 kT for a thickness of 5 nm. If $R<\delta$, film rupture occurs for a different reason. On reaching one side of the soap film, the particle may partly dissolve to release a surface-active agent that tends to spread at the air–liquid interface. The particle will then be surrounded by a region containing an excess of surfactants, creating a surface-tension gradient and a corresponding Marangoni (surface-tension-driven) flow in order to restore a uniform surfactant concentration at the film surface. This surface flow in turn couples to the inner fluid of the soap film, and tends to thin out the film, causing its rupture. Silicone-oil droplets or glass particles covered with polydimethylsiloxane chains are well-known antifoaming agents. The interfacial tensions of the surface-active species in these reagents are among the lowest of all organic materials, ensuring that they spread even on films of organic oils. Antifoaming agents are used, for example, in lubricating oils for car engines. As oil is constantly circulated around the pistons and the crankshaft, it incorporates considerable amounts of air, and thus has a tendency to form a foam, whereas for efficient lubrication the oil film must stay continuous. The appearance of gas–liquid interfaces also reduces the cooling capacity of the lubricant, creating hot spots in the engines. Various kinds of polymeric antifoaming agents are presently used in the petroleum industry.

Unconventional colloids

The colloidal systems discussed so far share the common property of forming connected networks. This property has led to many industrial applications, ranging from household items to road and building construction. We will now consider some of the more exotic kinds of colloidal system, such as lyotropic and thermotropic liquid crystals and two-dimensional amphiphile monolayers. On the whole, the possible applications of these materials are yet to be realized; but many of these are nevertheless particularly novel and appealing.

This is the case, for example, for Langmuir films (ordered layers of oriented amphiphiles), which, when transferred onto an appropriate solid substrate, may become used in molecular electronics, and already show promise as chemical sensors. Block copolymers provide the possibility of creating new types of polymer alloy. Lyotropic liquid crystals will probably find applications in cosmetics, pharmacology and drug delivery, and perhaps in tertiary oil recovery. Tubules formed from diacetylenic phospholipid liquid-crystal phases have a very broad range of possible uses ranging from components of cathode arrays (as used in electronic display panels) to anticorrosive paints. Our aim here is to describe the structures that underlie the key properties of these systems.

Block copolymers. These are fabricated by using a variation of the anionic polymerization technique[48]. A polymeric sequence of 'type A' monomers is first synthesized, and the 'living' (functionally active) end of this polymer is used as the initiator for the polymerization of a second sequence composed of monomers of type B. The active end of the B sequence is capped once it has reached its desired length. More complicated macromolecular structures can also be synthesized, such as triblocks (ABA or BAB) or 'star' blocks. Here we will be concerned only with diblocks.

The versatility of these systems results from the fact that the polymers that serve as the building blocks of the final structures are of mesoscopic size: molecular interactions are manifest only through the Flory interaction parameter χ between monomers A and B[19]. A negative value of χ indicates an attraction between A and B, positive χ indicates repulsion. The behaviour of these systems is therefore controlled solely by the numbers of each type of monomer, n_A and n_B, and χ, which includes both enthalpic and entropic contributions.

Here we consider the case $\chi>0$. At high temperatures, translational entropy dominates and a totally disordered state is obtained in which there is a uniform distribution of A and B segments. As the temperature is lowered, enthalpic effects become more important and the A and B subchains therefore tend to segregate. Neutron and X-ray scattering reveal a tendency towards segregation at a well defined wavelength corresponding to essentially twice the copolymer's natural radius of gyration $R_g=(n_A+n_B)^{1/2}$, as predicted theoretically[49].

On further cooling, this tendency towards segregation manifests itself by the appearance of permanently segregated structures at a certain transition temperature. Close to this temperature, the difference $\Delta\rho$ between the (properly weighted) monomer densities ρ_A and ρ_B is small: the system may then be described by a power expansion in $\Delta\rho$. This regime is called the 'weak' segregation limit. The transition temperatures can be

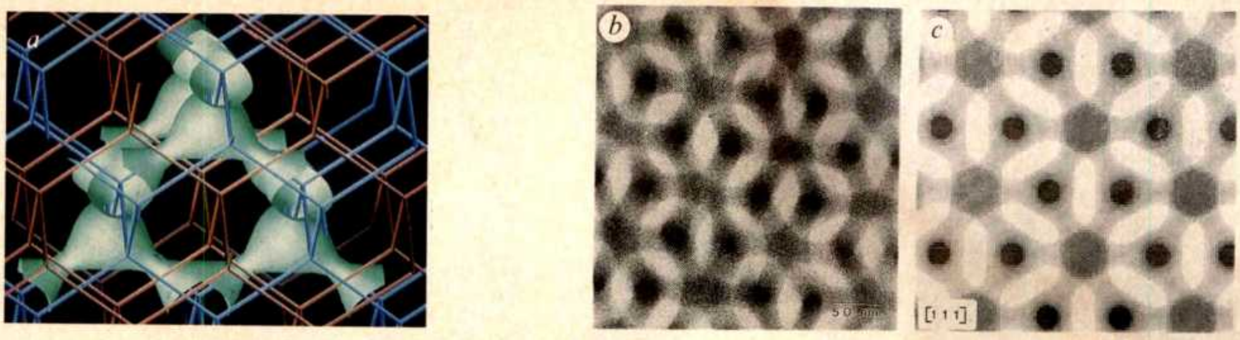


FIG. 4 Double-diamond structure. *a*, Real-space representation. *b*, Electron micrograph of a polystyrene–polyisoprene mixture. *c*, Computer-generated cross-section of the real-space structure (courtesy Thomas *et al.*[56]).

calculated uniquely in terms of n_A, n_B and χ (refs 49, 50), and these theoretical predictions compare reasonably with experiments. Four types of ordered phase have been observed: lamellar, columnar (columns of A surrounded by B if $n_A > n_B$, and vice versa), body-centred cubic, and an unusual bicontinuous 'double-diamond' structure that we describe below.

Lowering the temperature further takes the system into the 'strong' segregation regime, in which the position of the A–B interface is well defined and its thickness t is small. More precisely, experiment and theory show that $t \sim a\chi^{-1/2}$ (independent of n for large n). For example, $t \sim 2$ nm for a polystyrene–polybutadiene diblock[51]. Describing the structure by a Fourier expansion would require the use of an impractical number of terms. It is more appropriate to consider the monomer distributions under the constraint that the A–B bond is pinned at an infinitely sharp interface ($t = 0$). Reducing the total interfacial energy in this way tends to pull these interfaces apart as far as possible. This can be achieved only by stretching the polymers, which costs elastic energy. The balance of the elastic forces acting on one polymer segment and the pressure gradient that drives the stretching is equivalent, in the continuum limit, to the equation of motion of a classical particle in an external potential[52,53]. At equilibrium, the copolymers are stretched significantly: the periodicity scales as $(n_A + n_B)^{2/3}$ instead of $(n_A + n_B)^{1/2}$ as in the weak-segregation limit. The mechanical analogy is also useful for calculating characteristics such as the bending and compressional moduli in the lamellar phase)[54,55].

One of the most striking features of copolymer structures is the existence of an ordered, bicontinuous double-diamond structure (Fig. 4). It was first observed in more complex systems (star block) and later in a diblock system (62–68% polystyrene/38–32% polyisoprene, by volume)[56]. In the latter, polyisoprene forms two interpenetrating (but distinct) diamond networks, and polystyrene fills the remaining space. The occurrence of this phase may be explained by assuming that the leading contribution to the total energy is the interfacial energy between A and B: the system then tends to minimize the interfacial area at constant volume fraction (A and B being essentially incompressible fluids). The surfaces satisfying this condition have a constant mean curvature. Such surfaces are a generalization of the so-called Schwartz surfaces[125], for which the mean curvature is strictly zero.

These ordered block copolymer structures may find many applications. For instance, one may form a porous material with a well defined pore size and good mechanical stability by selectively dissolving one of the polymers. Block copolymers are also prototypes of amphiphilic molecules, facilitating the mixing of incompatible polymers A and B in the same way that short-chain surfactants allow the formation of stable oil–water emulsions. To this end, copolymers are extremely important for the development of polymer alloys. A technical difficulty arises, however, from the very slow diffusion of the long copolymer molecules through a polymer melt; this may prevent the thermal equilibrium configuration, which minimizes the free energy of the system, ever from being reached in practice. Current developments focus on the synthesis of copolymers directly at the interface, or the use of shear-mixing techniques to enhance polymer transport.

Mesoscopic scales in thermotropic liquid crystals. Low-molecular-weight thermotropic liquid crystals usually order on molecular scales, because of the short-range nature of the interactions. In some cases, however, they may develop larger-scale structures. Mesophases made of low-molecular-weight thermotropic liquid crystals are not considered traditionally as colloidal systems even though they may involve spatial scales in the relevant range, according to our earlier definition. The reason for this is more historical than fundamental, and we choose to describe a few such systems that are ordered at colloidal scales in view either of their similarity with other colloidal systems (this is the case for the so-called 'blue phases') or of their novelty (for example, 'incommensurate' smectics and the twist grain-boundary phases).

Chirality. Chirality of organic compounds normally results from the presence of an asymmetric carbon atom in the molecule. The consequences of chirality on mesoscale structures are illustrated most conveniently by considering a nematic phase composed of, say, elongated molecules that are parallel to each other on average and have their centres of gravity distributed at random.

Consider the helical distortion shown in Fig. 5*a*. If the nematic is made of enantiomorphic molecules (possessing mirror symmetry), there is no preference for one helical sense over the other, and a series expansion of the energy F in terms of the gradient of the (average) molecular direction θ_y must be quadratic to maintain this symmetry:

$$F \sim (\partial\theta_y/\partial x)^2$$

If, on the other hand, the constituent molecules are chiral, the sign of the helical distortion does matter: the free-energy expansion cannot be symmetrical in $\partial\theta_y/\partial x$, and the lowest-order term is linear[57]:

$$F \sim -q_0(\partial\theta_y/\partial x) + \tfrac{1}{2}(\partial\theta_y/\partial x)^2$$

Minimization of this expression gives the equilibrium condition $q_0 = \partial\theta_y/\partial x$. Thus, in its ground state a chiral nematic is not homogeneous but exhibits a helical precession: this is called a cholesteric phase. The relationship between nematics and cholesterics has been long recognized[58].

Experimentally, the pitch $2\pi/q_0$ of the helix often turns out to be in the visible range and this confers to the cholesteric striking irridescent colours by Bragg reflection. Because of the temperature dependence of the pitch, cholesterics are used as surface temperature sensors. The irridescence of some beetle cuticles stems from the same origin[59]. Neither the sign nor the order of magnitude of q_0 can be easily related to the molecular structure. The fact that the pitch is in the micrometre range indicates that chirality is poorly coupled to the long-range molecular order in the structure. For strong coupling the pitch is much shorter: this is the case, for example, with amino acids, for which the structure is directly related to molecular packing constraints.

Cholesterics are twisted in only one dimension (Fig. 5*a*). Other configurations are possible, however, that locally allow twisting in all directions perpendicular to a given molecular orientation (Fig. 5*b*). Such configurations, called double-twist structures, are energetically favoured close to the cholesteric–isotropic transition. Although double-twist structures cannot be space-filling, triply periodic (that is, crystalline) structures can be formed from intertwined double twists. The latter structures necessarily incorporate defects such as disclinations, and as a result they can exist only in a narrow temperature range close to the cholesteric–isotropic transition. They are called blue phases[60], after the colour seen under a polarizing microscope. In the absence of external magnetic or electric fields, body-centred cubic and simple cubic structures are the only ones observed. These crystals are unusual in being defined by a modulation not of mass density but of orientation.

The description of blue phases in terms of a network of double-twist cylinders is equivalent to the strong-segregation limit for copolymers[61]. An analogue of the weak-segregation limit (described in terms of Landau and Fourier expansions of the direction modulations[62,63]) has also been observed and demonstrates the importance of biaxiality. The frustration of preferred alignments in cholesteric phases can be relieved in theory by embedding the structures in a space of higher dimensionality[61]. Although not realizable in practice, this permits the structures of the blue phases to be discussed in purely geometric terms.

Natural cholesteric structures are fairly widespread. Dissolving the mineral component of bones leaves a collagen network that exhibits a typical blue-phase structure with identifiable double twists. Collagen in the derma of annelides also adopts a double-twist conformation[64]. These structures are probably not equilibrium ones, however.

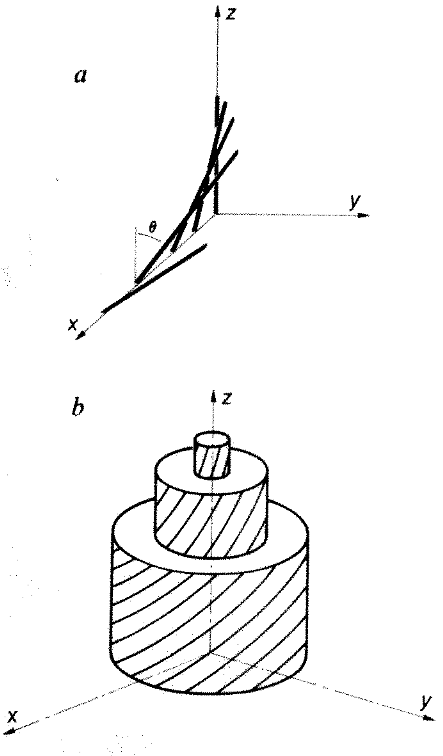


FIG. 5 *a*, One-dimensional cholesteric twist in a nematic liquid crystal. The structure is translationally invariant in the *y* direction. *b*, Double-twist structure: the molecular direction twists away from the *z* axis in all directions.

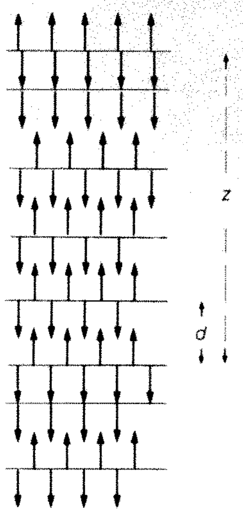


FIG. 6 Simplified representation of a smectic incommensurate phase. The period *z* is in general incommensurate with *d*. Arrows represent the molecular dipoles.

When a cholesteric undergoes a phase transition to a smectic A phase (in which molecules lie, on average, perpendicular to the layers), the pitch usually becomes infinite and the helical distortion disappears because of an incompatibility between the simultaneous existence of layers and twists. The cholesteric–smectic A transition can be described in terms similar to the normal-state–superconducting phase transition[65], whereby the exclusion of twist by formation of layers is analogous to the Meissner effect (exclusion of magnetic fields in the superconducting state), and dislocations are analogous to Abrikosov vortices. But superconducting states may also be of type II, in which magnetic fields greater than a threshold value H_{c1} may penetrate through periodic arrays of vortices. The analogue of this phase in the smectic A state of liquid crystals has recently been found experimentally and theoretically[66,67]. The structure, composed of screw dislocation walls, is more complicated than the type II superconductor's vortex state in that it incorporates a 'global' twist.

Incommensurability. Some liquid-crystal systems tend spontaneously to form layers with two incommensurate lengths, even though they contain only one type of molecule. Polar compounds, for example, tend to develop smectic phases with two different periodicities, one corresponding to the molecular length l and another corresponding to a molecular pair of length l' (usually $l < l' < 2l$). The molecular pair involves partial overlap of the molecules, which are usually antiparallel because of either dipolar or steric interactions. It is often not possible, however, to satisfy simultaneously both steric and dipolar constraints. Of the many consequences of this frustration[68-71], we outline here two that lead to structures containing a single, large length scale.

The first is an incommensurate smectic. If $l' \approx 2l$ (that is, the molecules do not overlap significantly), then for certain values of external parameters such as temperature, the system adopts a structure in which large conventional smectic domains of size $\sim l^2/(l'-l)$ are separated by smaller zones (of the order of a few times l) of defects (Fig. 6). In the large domains, the monolayer and bilayer lattices adopt a 'compromise' layer thickness d whereas in the defect zone one of the lattices undergoes a shift by an angle π with respect to the other[72]. Such a structure has been found experimentally (ref. 73). Frustration in the system may also lead to incommensurability through the development of a modulation parallel to the layers (Fig. 7)[74].

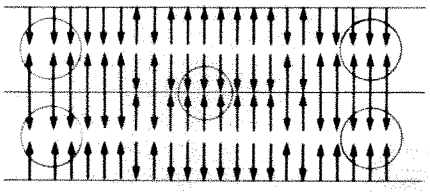


FIG. 7 Rectangular centred structure of the two-dimensional incommensurate smectic phase. The structure is translationally invariant perpendicular to the figure. The circles are guides to the eyes.

The colloidal length scale resulting from frustration now runs parallel to the layers, although the incommensurability is along the normal[69]. The so-called $P_{\beta'}$ phase of some lecithin-water systems bears much resemblance to tilted versions of this phase.

Lyotropic systems. Consider a two-component solution composed of a (small) volume fraction ϕ of amphiphilic molecules in a polar or non-polar solvent (in the latter case, the role of hydrophobic and hydrophilic groups is permuted). For simplicity, we consider water as the solvent. If the hydrophilic head creates about the same degree of steric hindrance as the aliphatic tail, the amphiphiles tend to aggregate into flat bilayers of thickness δ, the hydrophilic heads screening water molecules from the hydrophobic tails (Fig. 8).

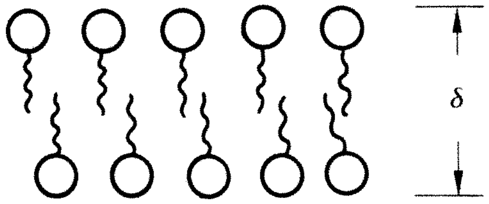


FIG. 8 Typical bilayer configuration of amphiphiles. The circles represent hydrophilic heads, and wavy lines represent hydrophobic aliphatic tails.

The simplest structure that one can imagine is a periodic stack of bilayers, with a period d such that $\phi = \delta/d$. The period d may reach mesoscopic scales if ϕ is small enough. Very large repeat distances, of the order of micrometres, have been observed experimentally[75]. But if bilayers tend to form at all, it should be favourable to make as many of them as possible, which raises the question of why such phases with large d are stable. As long-range van der Waals forces give rise to attractive interactions[5] in this configuration, large values of d can be obtained only if there is an additional repulsive interaction between layers. This is the case if the bilayers are electrically charged (that is, when the hydrophilic heads ionize): with ultra-pure water, the repulsive term varies as kT/bd^2 per unit volume (here b is the Bjerrum length, of the order of a few nanometres in aqueous systems). This repulsion outweighs the van der Waals attraction at large d, stabilizing the structure. Adding salt introduces screening, and one would then expect the period to stabilize at the value $d \sim \lambda$ (λ being the Debye screening length). But this is not the case: the relation $d \sim \delta/\phi$ still holds. Moreover, even in the absence of electrostatic interactions, large periodicities can be found.

The reason for this is that there is also a repulsion of entropic origin[76]. A single bilayer fluctuates with a mean-square displacement $\langle u^2\rangle$ which is proportional to the square of the linear size of the bilayer. In a periodic stack, these fluctuations are suppressed if $\langle u^2\rangle \sim d^2$, whereupon the bilayer loses an entropy contribution to the free energy. This entropy term becomes manifest as an apparent repulsion, proportional to $(kT)^2/\kappa d^3$ per unit volume (κ is the bending modulus of the bilayer).

X-ray scattering seems to bear out this simple explanation. It has been long known[77,78] that two-dimensional (layered) systems cannot exhibit true long-range order because thermal fluctuations tend to destroy it at any non-zero temperature. As a result, the X-ray scattering from such systems does not show resolution-limited Bragg peaks, but rather power-law divergences in intensity I at certain wavevectors q of the type[57] $I \sim 1/|q-q_0|^{2-\eta}$, where $q_0 = 2\pi/d$ and η is an exponent that depends on both the curvature and compressional modulus of the lamellar phase. For large d, η tends towards a universal value (that is, independent of the specific characteristics of the system), and experiment can then be compared with theory without any adjustable parameters. Such experiments have been performed and support these predictions[79]. The cross-over to the electrostatic-repulsion regime has also been studied. As these systems are strongly fluctuating, refined treatments involving field-theoretical techniques such as the renormalization group are needed to fully understand their behaviour[80,81]. They confirm the existence of the scaling regimes described above, but also show that, beyond a critical value of the Hamaker constant, one cannot swell a lamellar phase to arbitrarily large periodicities.

Consider now the thermally activated appearance of a hole in one of the layers (Fig. 9a). This may occur without exposing aliphatic tails to the water, at the expense of forming a highly curved surface, costing an energy of the order of $\kappa r/\delta$, where r is the radius of the pore. If r is to be significant, the cost is very large relative to kT and the occurrence of such a pore is highly unlikely. But if the pore connects two sheets (Fig. 9), the cost in curvature energy is much less (of order κ for $r \sim d$). As κ is only a few times kT, such connecting pores are fairly probable. Another circumstance favours their formation at high dilution: the curvature modulus is renormalized to smaller and smaller values as d increases, when the length scale is renormalized to d. In other words, small-scale undulations favour the appearance of large ones[82-84]. As a result, beyond a certain separation d_0 lies a regime in which the energy cost of hole formation is outweighed by entropy gains, and they proliferate. They can then either build up a regular array of connecting pores (Fig. 10) or simply destroy the long-range order of the sheets, giving rise to a 'sponge' phase. Because the topology of the system changes, one must distinguish between mean and gaussian curvatures. Each point of a surface is characterized by two principal radii of curvature, R_1 and R_2; the mean curvature is $(1/R_1 + 1/R_2)$, and the gaussian curvature is $1/R_1R_2$. According to Gauss's theorem[85], the integral of the gaussian curvature is a constant for a given topology. Depending on the details of the interactions, the system may form 'crystals' of droplets created by regular arrays of holes, or connected structures.

A sponge phase consists of a surface made of bilayers with a typical lateral size proportional to d_0, connected at random but avoiding both pores within the bilayers (see Fig. 9a) and seams (line defects at which three bilayers meet). These constraints are the result of the very high curvature energies of seams and pores. With these assumptions, the random bilayer surface can be shown to divide space into two distinct subspaces A and B, which may or may not be globally equivalent (symmetric or non-symmetric sponges respectively)[86]. Light-scattering experiments have revealed fluctuations linked to the transition from the symmetric to the non-symmetric state[87]. If the sheets are imperfect (such that fluctuating pores in a single

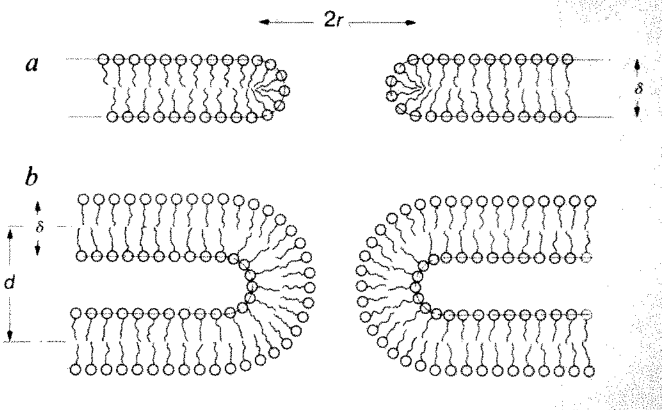


FIG. 9 a, Pore in a single amphiphilic bilayer. b, Pore connecting two bilayers.

sheet already destroy the difference between interior and exterior), the situation is far more complex and its description requires the use of gauge field theories of the kind used in particle physics[88]. The net result is a subtle interplay between phases in which the notions of line tension and/or interfacial tension vanish, and others in which they do exist at large scales. It seems that experimental systems have the potential to realize all these cases.

So far, we have described systems in which bilayers, considered as surfaces, 'float' in a single solvent. Similar ideas apply to immiscible (say, oil-water) mixtures separated by a single layer of surfactants and co-surfactants (such as a sodium dodecyl sulphate-pentanol mixture). In these systems, called microemulsions, the curvature energy of the films again plays an important role. By describing fluctuations responsible for entropic repulsion in terms of renormalization of the curvature modulus κ, one can define an in-plane correlation length $\xi \sim \exp(\kappa/kT)$ beyond which the interface has lost any memory of its previous orientation (this length is basically the same as the sheet separation d_0 beyond which connecting pores become highly probable). One may again find regimes in which the stable phases are fluctuating bicontinuous structures, droplets of oil in water, droplets of water in oil, or lamellar structures[89]. The description is essentially the same as that of lyotropic bilayers, except for the existence of two independent volume fractions (that of the surfactant and that of oil or water) and the inherent breaking of the interior-exterior symmetry. A bicontinuous phase has been seen experimentally[90]. Such phases are potentially important in the oil industry because they provide a way to dissolve oil in water using only small amounts of surfactants.

The biologically important phospholipids tend to form stiffer layers which cannot swell easily. Addition of cholesterol stiffens the layers still further (κ can be as large as 50 kT), whereas the addition of alcohols increases the flexibility, allowing swelling to occur. Ordered structures such as bicontinuous cubic phases have been known for a long time[91]. In these phases, thermal fluctuations play a less essential role than in those described previously. The observed structures can be classified using a geometrical approach. In general, hydrophilic heads do not experience the same steric hindrance as hydrophobic tails, so packing amphiphilic molecules together on surfaces causes frustration. This frustration can be removed for structures constructed mathematically on a four-dimensional sphere[92]. Unfolding the curved four-dimensional space on a tangential three-dimensional euclidean space produces all the known structures, as well as predicting a few more.

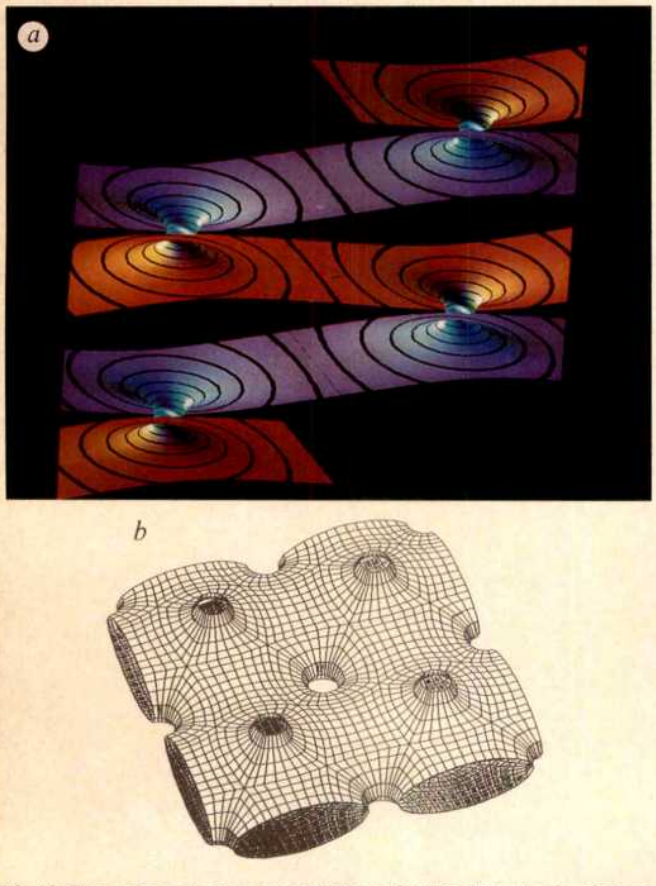


FIG. 10 Three-dimensional representations of ordered structures of pores connecting bilayers. *a*, A minimal surface connecting sheet (courtesy Thomas *et al.*[56]). *b*, A triplyperiodic minimal surface (courtesy S. Leibler & A. Maggs).

It is interesting to consider whether it is possible to prepare colloidal lyotropic systems using liquid-crystalline solvents. The case of a highly swollen lamellar phase in a nematic solvent has been discussed recently[93]. Here, molecular orientational fluctuations are responsible for an attractive interaction between the lamellae when they are symmetric, just as polarization fluctuations give rise to van der Waals forces. As a result, nematic solvents should not be able to swell symmetric layers. Asymmetric layers can be swelled, however, but this case seems more difficult to achieve experimentally.

Vesicles and tubules. If one brings into contact essentially pure water with a lamellar phase of phospholipid molecules, extensive swelling of the lamellae is often observed. They bulge into the fluid and eventually close up to form vesicles a few tens of micrometres in size. The vesicles comprise one or several bilayers surrounding a pocket of fluid. They are roughly the same size as living cells, but are immensely simpler and therefore provide a convenient model system with which to study the parameters that control the morphology and shape fluctuations of such highly deformable closed systems. Curvature energy is clearly important in determining the behaviour of lyotropic layers, but this is not the whole story: as the vesicle is closed, there is also in general a pressure difference between the interior and exterior. Fluid exchange through the membrane is usually so slow that the trapped fluid volume provides a convenient measure of this pressure difference. On the experimental timescale of a few hours, the number of molecules in the film stays constant, and as fluctuations in the area per molecule are small, the vesicle's surface area remains constant to a good approximation. Interfacial energies then play no part in determining the shape.

Furthermore, it is possible to confine particular species to just one side of the membrane: this breaks the interior-exterior symmetry. Just as cholesterics have a preferred twist, one finds that such membranes have a preferred curvature, which may not coincide with that dictated by the surface-to-volume ratio.

With these ingredients at hand, one can examine the stability of typical morphologies of red blood cells[94], and calculate the conditions under which 'budding' (breaking off of new vesicles) or 'fusion' of vesicles occurs[95]. These latter shape changes are greatly facilitated if the chemical composition of the film can be varied laterally[80]. This approach may provide the first steps in understanding exocytosis and endocytosis (in which closed vesicles containing macromolecules fuse with or detach from cell membranes, respectively). Small shape fluctuations can be described analytically, allowing the membrane flexibility to be determined[96,97], but in general the problem is not tractable analytically. A simulation scheme has been devised recently that allows one to consider anharmonic fluctuations of arbitrary shapes (A. Maggs, S. Leibler and L. Drouf, personal communication). Direct manipulation of vesicles with micropipettes allows one to measure their main characteristics, such as bending modulus and lateral compressibility[98].

The calculations reproduce the shapes of red blood cells, but they clearly do not include all of their physics. In particular, the spectrin network which spans the inner side of the membrane is not taken into account. This net corresponds to our definition of a gel and, being anchored to the membrane by proteins, is two-dimensional. Computer simulation has been used to study such polymerized membranes. They can reproduce the observed discocyte and stomatocyte shapes as well as their fluctuations and elastic properties[99]. One of the interesting questions posed by polymerized membranes is whether they lie more or less flat or form a compact 'crumpled' phase. The current consensus is that fluid membranes are always crumpled whereas (self-avoiding) polymeric ones are always flat irrespective of temperature[100].

In-plane ordering of the molecules may exhibit a rich diversity of behaviour, of which we choose here to discuss only one particular aspect. Suppose that the molecules are tilted cooperatively with respect to the membrane normal. This is the case, for example, for some diacetylenic lecithins below about 50 °C (ref. 101). On a closed surface, which has the topology of a sphere, the tilt direction cannot be homogenous: two defects are inevitable at the north and south poles of the globe. If the long-range orientational order is strong enough, these defects cost too much energy and the structure opens spontaneously to form a hollow cylinder (J. Prost, S. Lieven, T. C. Lubensky and S. Ramaswamy, manuscript in preparation). It seems likely that this mechanism plays a role in the formation of hollow tubules from vesicles made of diacetylenic lecithins. Because these systems are chiral, the process involves the intermediate structure of a helical ribbon[101]. Such tubules are polymerizable and can be metal-coated. This suggests a large number of potential applications, including anti-corrosive and high-dielectric paints and blood substitutes. The fabrication of polymeric vesicles could also be useful for drug delivery[102].

Langmuir films. Amphiphilic molecules such as long-chain fatty acids and phospholipids spread readily at the air–water interface with their polar head, generally a carboxylic acid group in the case of fatty acids, in contact with water and their aliphatic long hydrocarbon chains pointing towards the gas phase. The thickness of the adsorbed layer corresponds to a single molecular length, of the order of 2 nm for a chain with 20 methylene units. The surface density can be varied by pushing the molecules together using a non-wettable barrier made of Teflon. At maximum compression, the area occupied per molecule is 0.18–0.2 nm^2, corresponding to a close-packed array of vertical chains in the all-*trans* configuration. The surface pressure exerted by the monolayer on the barrier is ~30-40 dyne cm^{-1}. As the surface density is gradually lowered, the monolayer undergoes a series of phase transitions from a solid phase (S) to a 'liquid-condensed' (LC), 'liquid-expanded' (LE), and eventually a gas phase (G)[103]. The LE–LC and LE–G transitions are first-order and thus characterized by plateaus in the surface pressure isotherms (Fig. 11). Not much is known about the structure of the LC, LE and G phases. Their fluidity implies the absence of long-range translational order, but recent X-ray surface-diffraction experiments suggest the possibility of long-range orientational ordering in the LC phase[104]. X-ray reflectivity[105-107] measurements give information on the electron density profile perpendicular to the interface. In the LC phase, the chains adopt the all-*trans* conformation, often tilted at angles varying from 0 to 30°. One significant development in this field is the recent experimental identification of crystalline structure in the two-dimensional solid phases[108]. Several such phases have been observed: hexagonal close-packed, distorted hexagonal, with or without tilt of the molecules (including several different tilt directions with respect to the lattice).

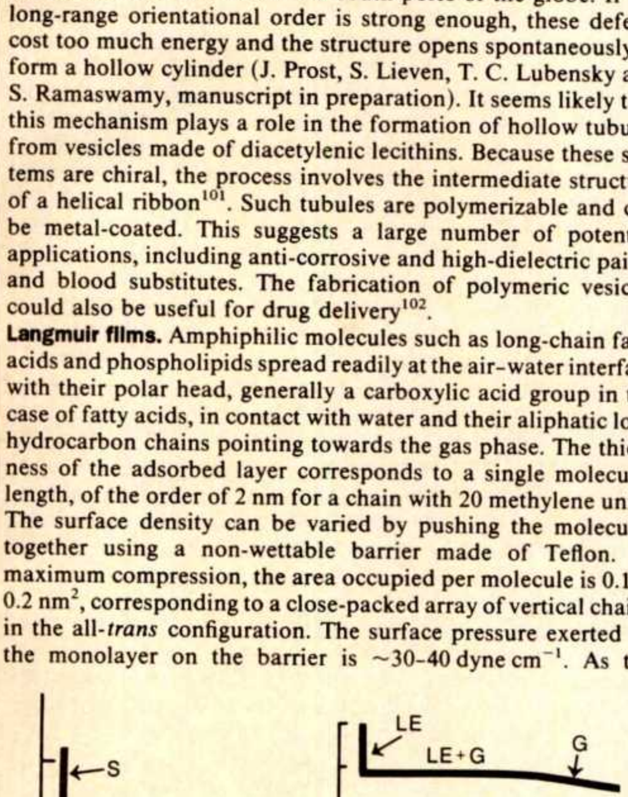


FIG. 11 Schematic isotherm for a Langmuir monolayer, a plot of surface pressure π against area per molecule. The inset shows the extension of the LE+G region at high areas and the transition to the single-phase gas region G.

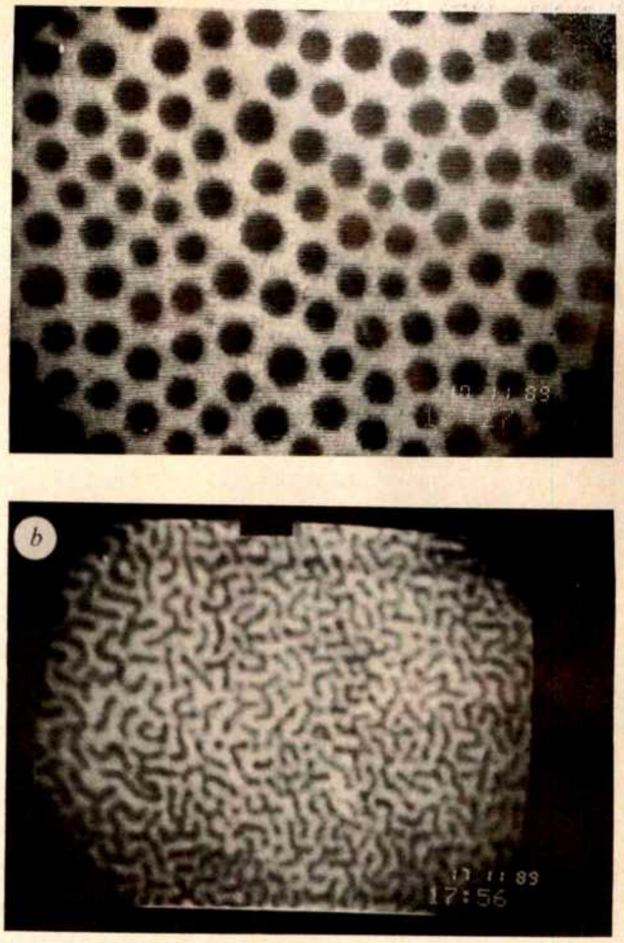


FIG. 12 *a*, Array of liquid-condensed islands interacting via long-range repulsive forces in a Langmuir film. *b*, The stripe structure formed as a result of strong electrostatic repulsion between domains in *a*. (Photographs by S. Akamatsu & E. To, Univ. Paris VI.)

The LC–LE and LE–G plateaus extend over large regions of the phase diagram, typically from 0.22 to 0.40 nm^2 per molecule for the LC–LE transition and from 0.60 to 20 nm^2 per molecule for the LE–G transition. In these regions, there is coexistence between two different phases and the surface fraction of one phase in the other is given by the lever rule along the tie lines.

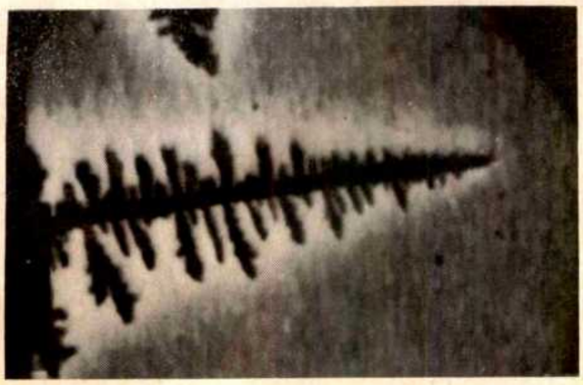


FIG. 13 'Dendritic' growth of a liquid-condensed domain in a Langmuir monolayer.

The monolayer is therefore heterogeneous on a macroscopic scale and interesting structures appear which have been revealed by epifluorescence microscopy. This technique involves doping of the monolayer with a small amount of a fluorescent probe[109,110]. The probe partitions unevenly between the phases, either because of its differential solubility or because of the intrinsic density differences, and thus distinguishes the coexisting phases. As the monolayer is compressed from the LE phase into the LE–LC two-phase region, continuous growth of domains of the LC phase is observed. As the LC side of the coexistence curve is approached, these domains reach diameters of several hundred micrometres (Fig. 12*a*).

This figure reveals several interesting features. First, although the domains are nucleated at random within the monolayer, they have a tendency to organize into a supramolecular crystal when their mean interdistance becomes comparable to their diameter. The formation of this crystal is the result of a marked repulsion between neighbouring domains, as is the case for charged colloidal particles. This repulsion is clearly electrostatic in origin, because the minimum approach distance decreases on adding salt to the subphase[112]. It is also a function of the domain size: repulsion is less pronounced between smaller domains. This effect is related to the component of the dipole moments of the surfactant molecules perpendicular to the interface. All the dipoles point in the same direction, so that each domain can be considered as a macro-dipole that repels its neighbours[113,114]. In some special cases, the dipole–dipole repulsion between two molecules is strong enough to deform the shape of each individual domain. A circular domain becomes distorted into an ellipse; eventually, the ellipses fuse in long alternating stripes of LC and LE phases with a periodicity of between one and ten micrometres. This is the so-called stripe structure[115] (Fig. 12*b*).

Second, the LC domains can sometimes exhibit striking patterns reminiscent of fractals or dendrites (Fig. 13)[116]. This highly unstable growth requires specific experimental conditions, such as fast compression of the monolayer and large viscosity of the newly formed LC phase. This instability has been attributed to the slow diffusion of impurities out of the growing domains and into the bulk LE phase. It is known that impurities induce supercooling effects and consequently slow down the growth of the more condensed phase. Exodiffusion, and therefore the growth rate, is proportional to the concentration gradient. If protuberances form at some points along the interface, the gradient is locally steeper and the growth accelerated, further increasing the amplitude of the distortion. This mechanism of unstable growth was first predicted by Mullins and Sekerka in the context of metallurgy[117]. The ramified patterns that result are not stable, however, and line tension, which tends to minimize the perimeter of the LE–LC interface, will eventually transform them into circles. Depending on the monolayer viscosity, this relaxation can take anywhere from minutes to days.

As the monolayer is expanded from the LE one-phase region into the LE–gas coexistence region, circular gas bubbles are formed. This is again a nucleation process, similar to that of the LE–LC transition. On further expansion, the gas bubbles grow and start to deform as they progressively fill the whole surface of the monolayer, leaving only narrow liquid regions between them[118] (Fig. 14). A polyhedral cell structure is obtained for average densities between 2 and 10 nm^2 per molecule. Polygon diameters vary from tens to hundreds of micrometres, with the average number of sides equal to six. Polygons are separated by strips of the continuous liquid phase of width 10–20 μm. Three strips meet at stable Plateau vertices with apical angles of 120°. When four strips meet, the arrangement is unstable and the cells rearrange themselves spontaneously. The driving force for this is the minimization of the total length of the strips, in order to reduce the line energy. Theory predicts that the long-time distribution $P(n, t)$ of polygons with n sides should be a universal function independent of t, whereas the average polygon area should grow linearly with time[119,120]. Recent experiments on the evolution of foam structures in Langmuir films have confirmed these expectations[121-123].

The topology of two-dimensional foams resembles that of the three-dimensional ones discussed earlier. Two-dimensional foams in Langmuir monolayers have the advantage of being free from the effects of drainage by gravity, as they are produced in a horizontal plane. They are therefore much more stable and their evolution can be studied over several days. Furthermore, the observations can be compared directly with numerical simulations, which would be prohibitively complex in three dimensions. One open question is why the width of the liquid strip seems to reach an equilibrium value. Two different explanations have been proposed, one static (based on the existence of long-range dipolar forces[122]) and the other hydrodynamic (based on the formation of line-tension gradients owing to adsorption of impurities at the liquid–gas interface[124]).

Concluding remarks

Despite the complexity of the systems that we have described, their structures are explicable in simple geometrical terms involving lines, surfaces and densities. This is meaningful precisely because the relevant length scales are significantly larger than molecular or monomeric. The concept of universality

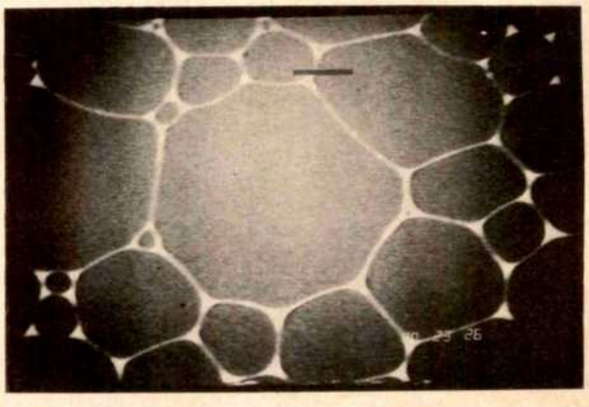


FIG. 14 Polygonal structure of a two-dimensional Langmuir foam in the gas–liquid coexistence region.

inheres to much of this description, and this principle is valuable in allowing us to consider the phenomena in a unified framework. It should not be forgotten, however, that the details of many natural phenomena rely on selectivity and specificity.

A balance between universality and specificity seems to be appropriate for developing further our understanding of colloidal systems. This is certainly true for the conception and preparation of new materials, but perhaps also for placing on more quantitative grounds the biological processes that occur at colloidal scales. A good example of this combination is provided by the elegant stereospecific chemistry that is possible on Langmuir films at the solid–liquid-condensed transition. On the theoretical side, there is a clear need for calculations of system-dependent quantities such as elastic moduli and transition temperatures at the same level of accuracy as has been achieved for block copolymers. This task is far from trivial, however.

Having focused this article on structures, we have essentially set aside dynamical phenomena. These are of paramount importance, and our understanding of them is developing fast. ☐

Jacques Prost is at the Ecole Supérieure de Physique et Chimie Industrielle de la Ville de Paris, Laboratoire de Physico-Chimie Théorique, 10 rue Vauquelin, 75231 Paris Cedex 05, France. Francis Rondelez is at the Université Pierre et Marie Curie, Laboratoire de Structure et Réactivité aux Interfaces, 11 rue Pierre et Marie Curie, 75231 Paris Cedex 05, France.

---

1. Graham, T. *Phil. Trans. R. Soc.* **151,** 183 (1861).
2. Einstein, A. *Ann. Phys.* **19,** 289–306 (1906).
3. Vanderhoff, J. W. & van de Hul, J. *J. macromolec. Sci. Chem.* **A7,** 679–687 (1973).
4. Tanford, C. *The Hydrophobic Effect* 2nd edn (Wiley, New York, 1980).
5. Israelachvili, J. N. *Intermolecular and Surface Forces* (Academic, London, 1985).
6. Kose, A., Ozaki, M., Takano, K., Kobayashi, Y. & Hachisu, S. *J. Colloid Interface Sci.* **44,** 330–338 (1973).
7. Pieranski, P. *Phys. Rev. Lett.* **45,** 569–572 (1980).
8. Murray, C. A. & Van Winkle, D. H. *Phys. Rev. Lett.* **58,** 1200–1203 (1987).
9. Pusey, P. N. *J. Phys. (Paris)* **48,** 709–712 (1987).
10. Pusey, P. N. & Van Megen, W. *Nature* **320,** 340–342 (1986).
11. Bibette, J., Roux, D. & Nallet, F. *Phys. Rev. Lett.* **64,** 2470–2473 (1990).
12. Van Winkle, D. H. & Murray, C. A. *Phys. Rev.* A **34,** 562–573 (1986).
13. Sood, A. K. in *Solid State Physics* Vol. 45 (eds Ehrenreich, E. & Turnbull, D.) 1–73 (Academic, New York, 1991).
14. Alder, B. J., Hoover, W. G. & Young, D. A. *J. Chem. Phys.* **49,** 3679 (1968).
15. De Gennes, P. G. *Adv. Colloid Interface Sci.* **27,** 189–209 (1987).
16. Asakura, S. & Oosawa, F. *J. chem. Phys.* **22,** 1255–1256 (1954).
17. Cabane, B. & Duplessix, R. *Colloid Surf.* **13,** 19–33 (1985).
18. Guo, X. H. & Chen, S. H. *Phys. Rev. Lett.* **64,** 2579–2582 (1990).
19. Flory, P. J. *Principles of Polymer Chemistry* (Cornell University Press, 1971).
20. De Gennes, P. G. *Scaling Concepts in Polymer Physics* (Cornell University Press, 1979).
21. Vidakovic, P. & Rondelez, F. *Macromolecules* **16,** 253–261 (1983); **17,** 418–425 (1984); **18,** 700–708 (1985).
22. Langevin, D. & Rondelez, F. *Polymer* **19,** 875–882 (1978).
23. Benoit, H. in *Physics of Finely Divided Matter* (eds Boccara, N. & Daoud, M.) 2–15 (Springer, Berlin, 1984).
24. Bastide, J. & Leibler, L. *Macromolecules* **21,** 2647–2649 (1988).
25. Candau, S. J., Bastide, J. & Delsanti, M. *Adv. Polym. Sci.* **44,** 27–71 (1982).
26. Bastide, J., Duplessix, R., Picot, C. & Candau, S. J. *Macromolecules* **17,** 83–93 (1984).
27. Tanaka, T. *Sci. Am.* **244,** 110 (1981).
28. Pappas, N. A. *Hydrogels in Medicine and Pharmacy* Vols 1 & 2 (CRC Press, Boca Raton, 1987).
29. Ramachandran, G. B. *Treatise on Collagen* Vol. 1 (Academic, New York, 1967).
30. Djabourov, M. *Contemp. Phys.* **29,** 273–287 (1988).
31. Porte, G., Appell, J. & Poggi, Y. *J. phys. Chem.* **84,** 3105 (1980).
32. Cates, M. E. & Candau, S. J. *J. Phys.* C **2,** 6862–6892 (1990).
33. Ott, A. *et al. J. Phys.* C **2,** 5907–5912 (1990).
34. Tachibana, T., Mori, T. & Hori, K. *Bull. Chem. Soc. Jap.* **53,** 1714–1719 (1980).
35. Terech, P., Volino, F. & Ramasseul, R. J. *J. Physique* **46,** 895–903 (1985).
36. Skoulios, A. *Adv. Colloid Interface Sci.* **1,** 79–110 (1967).
37. Witten, T. A. *J. Phys. (Paris)* **49,** 1055–1063 (1988).
38. Witten, T. A. *J. Phys.* C **2,** SA1–SA8 (1990).
39. Yen, T. F. in *Proc. 178th Meet. Am. Chem. Soc. Div. Petrol. Chem.* (ed. Bunger, J. W.) (American Chemical Society, Washington, DC, 1981).
40. Brûlé, B., Ramond, G. & Such, C. *Bull. Liaison du Laboratoire des Ponts et Chaussées* **148,** 69 (1987).
41. Mysels, K. J., Shinoda, K. & Frankel, S. *Soap Films. Studies of their Thinning* (Pergamon, New York, 1959).
42. Belorgey, O. & Benattar, J. J. *Phys. Rev. Lett.* **66,** 313–316 (1991).
43. Bikerman, J. J. *Foams* (Springer, Berlin, 1973).
44. Adamson, M. *Physical Chemistry of Surfaces* 5th edn (Wiley, New York, 1990).
45. Kahlweit, M. *Adv. Colloid Interface Sci.* **5,** 1–35 (1975).
46. Plateau, J. A. F. *Statique Expérimentale et Théorique des Liquides Soumis aux Seules Forces Moléculaires* (Gauthier-Villars, Paris, 1873).
47. Wilson, A. J. *Foams: Physics, Chemistry and Structure* (Springer, Heidelberg, 1990).
48. Szwarc, M., Levy, M. & Milkovich, R. *J. Am. chem. Soc.* **78,** 2656–2657 (1956).
49. Leibler, L. *Macromolecules* **13,** 1602–1617 (1980).
50. Bates, F. S. & Frederickson, G. H. *A. Rev. phys. Chem.* **41,** 525–557 (1990).
51. Helfand, E. & Wasserman, R. *Macromolecules* **9,** 879–888 (1976).
52. Semenov, A. N. *Zh. Exp. theor. Phys.* **88,** 1242–1256 (1985); Engl. transl. in *Sov. Phys. JETP* **61,** 733–742 (1985).
53. Milner, S., Witten, T. A. & Cates, M. E. *Europhys. Lett* **5,** 413–418 (1988).
54. Milner, S. T. *Europhys. Lett.* **7,** 695–699 (1988).
55. Milner, S. T. & Witten, T. A. *J. Phys. (Paris)* **49,** 1951–1962 (1988).
56. Thomas, E. L., Anderson, D. M., Henkee, C. S. & Hoffmann, D. *Nature* **334,** 598–601 (1988).
57. De Gennes, P. G. *The Physics of Liquid Crystals* (Clarendon, Oxford, 1972).
58. Friedel, G. *Annales de Physique* **18,** 273–474 (1922).
59. Bouligand, Y. *Tiss. Cell* **4,** 189 (1972).
60. Stegemeyer, H., Blumel, Th., Hiltrop, K., Onusseit, H. & Porsch, F. *Liq. Cryst.* **1,** 3–28 (1985).
61. Wright, D. C. & Mermin, N. D. *Rev. mod. Phys.* **61,** 385–432 (1989).
62. Hornreich, R. M. & Shtrikman, S. *Z Phys.* B **68,** 369–373 (1987).
63. Belyakov, V. A. & Dimitrienko, V. E. *Usp. Fiz. Nauk.* **146,** 369–415 (1985).
64. Gael, F. *J. Phys. Colloq.* **C7 (31)** 169–182 (1990).
65. De Gennes, P. G. *Solid St. Comm.* **10,** 753–756 (1972).
66. Renn, S. R. & Lubensky, T. C. *Phys. Rev.* A **38,** 2132–2147 (1985).
67. Goodby, J. W., Waugh, M. A., Stein, S. M., Chin, E. & Pindak, R., *Nature* **337,** 449–452 (1989).
68. Prost, J. *Adv. Phys.* **33,** 1–46 (1984).
69. Pommier, J., Barois, P. & Prost, J. in *Solitons in Liquid Crystals* (eds Ian, L. & Prost, J.) (Springer, Berlin, 1990).
70. Park, Y., Lubensky, T. C., Barais, P. & Prost, J. *Phys. Rev.* A **37,** 2197–2213 (1988).
71. Garland, C. *Proc. NATO Adv. Workshop on Quasi-Crystals, Liquid Crystals and Incommensurate Insulators* Reveza, Greece (Plenum, New York, 1989).
72. Prost, J. *Proc. Conf. Liquid Crystals of One and Two Dimensional Orders* Garmisch Partenkirchen, 125–145 (Springer, Berlin, 1980).
73. Ratna, B. R., Shashidar, R. & Prasad, S. K. *Phys. Rev. Lett.* **53,** 2141–2144 (1984).
74. Levelut, A. M., Tarento, R. J., Hardouin, F., Achard, M. F. & Sigard, G. *Phys. Rev.* A **24,** 2180–2186 (1981).
75. Larché, F., Appell, J., Porte, G. Bassereau, P. & Marignan, J. *Phys. Rev. Lett.* **56,** 1700–1703 (1986).
76. Helfrich, W. *Naturforsch. Z.* **33a,** 305–315 (1978).
77. Landau, L. D. *Phys. Z. Sov. Union* **2,** 26 (1937).
78. Peierls, R. E. *Ann. Inst. Henri Poincaré* **5,** 177 (1935).
79. Roux, D. & Safinya, C. R. *J. Phys. (Paris)* **48,** 673–680 (1987).
80. Leibler, S. in *Statistical Mechanics of Membranes and Surfaces* (eds Nelson, D., Piran, T. & Weinberg, S.) 46–101 (World Scientific, Singapore, 1989).
81. Lipowsky, R. & Leibler, S. *Phys. Rev. Lett.* **56,** 2541–2544 (1986).
82. Helfrich, W. *J. Physique (Paris)* **46,** 1263–1268 (1985).
83. Peliti, L. & Leibler, S. *Phys. Rev. Lett.* **54,** 1690–1693 (1985).
84. Golubovic, L. & Lubensky, T. C. *Phys. Rev.* B **39,** 12110–12133 (1989).
85. Boothby, W. M. *An Introduction to Differential Manifolds and Riemannian Geometries* (Academic, New York, 1986).
86. Roux, D. & Cates, M. in *Dynamics and Patterns in Complex Fluids* (eds Onuki, A. & Kawasaki, K.) 19 (Springer, Berlin, 1990).
87. Roux, D. *et al. Europhys. Lett.* **11,** 229–234 (1990).
88. Huse, D. A. & Leibler, S. *Phys. Rev. Lett.* **66,** 437–440 (1991).
89. De Gennes, P. G. & Taupin, C., *J. phys. Chem.* **86,** 2294–2304 (1982).
90. Auvray, L., Cotton, J. P., Aubert, R. & Taupin, C. *J. Physique (Paris)* **45,** 913–928 (1984).
91. Luzzati, V., Mariani, P. & Gulik-Krzywicki, T. in *Physics of Amphiphilic Layers* (eds Langevin, D. & Meunier, J.) 131–137 (Springer, Berlin, 1987).
92. Charvolin, J. & Sadoc, J. F. *J. de Physique (Paris)* **48,** 1559–1569 (1987).
93. Ajdari, A., Peliti, L. & Prost, J. *Phys. Rev. Lett.* (in the press).
94. Deuling, H. J. & Helfrich, W. *Biophys. J.* **16,** 861–868 (1976).
95. Lipowsky, R. *Nature* **349,** 475–481 (1991).
96. Bivas, E., Hanusse, P., Bothorel, P., Lalanne, J. & Aguerre-Chavriol, C. *J. de Physique (Paris)* **48,** 855–867 (1987).
97. Sackmann, E., Fischer, A. & Frey, W. in *Physics of Amphiphilic Layers* 25–37 (Springer, Berlin, 1987).
98. Waugh, R. & Evans, E. *Biophys. J.* **26,** 115–131 (1979).
99. Leibler, S. & Maggs, A. *Proc. Natn. Acad. Sci.* 6433–6435 (1990).
100. Nelson, D. in *Statistical Mechanics of Membranes and Surfaces* (eds Nelson, D., Piran, T. & Weinberg, S.) 1–16 (World Scientific, Singapore, 1989).
101. Georger, J. H. *et al. J. Am. chem. Soc.* **109,** 6169–6175 (1987).
102. Schmur, J. M. *et al. Thin Solid Films* **152,** 181–190 (1987).
103. Mohwald, H. *A. Rev. phys. Chem.* **41,** 441 (1990).
104. Kjaer, K., Als-Nielsen, J., Helm, C. A., Laxhuber, L. A. & Mohwald, H. *Phys. Rev. Lett.* **58,** 2224–2227 (1987).
105. Schlossman, M. L. *et al.* in *Mat. Res. Soc. Proc. Macromolecular Liquids* (eds Safinya, C. R., Safran, S. A. & Pincus, P. A.) 351 (Materials Research Society, Boston, 1989).
106. Helm, C. A., Möhwald, H., Kjaer K. & Als-Nielsen, J. *Europhys. Lett.* **4,** 697–703 (1987).
107. Daillant, J., Bosio, L. & Benattar, J. J. *Europhys. Lett.* **12,** 715–720 (1990).
108. Lin, B., Shih, M. C., Bohanon, T. M., Ice, G. E. & Dutta, P. *Phys. Rev. Lett.* **65,** 191–194 (1990).
109. Von Tscharner, V. & McConnell, H. M. *Biophys. J.* **36,** 409 (1981).
110. Lösche, M., Sackman, E. & Möhwald, H. *Berl. Bunsen. Ges. Phys. Chem.* **87,** 848 (1983).
111. Peters, R. & Beck, K. *Proc. natn. Acad. Sci. U.S.A.* **80,** 7183–7187 (1983).
112. McConnell, H. M., Tamm, L. K. & Weiss, R. M. *Proc. natn. Acad. Sci. U.S.A.* **81,** 3249–3253 (1984).
113. Andelman, D., Brochard, F. & Joanny, J.-F. *J. chem. Phys.* **86,** 3673–3681 (1987).
114. McConnell, H. M. & Moy, V. T. *J. phys. Chem.* **92,** 4520–4525 (1988).
115. Hekl, W. M. & Möhwald, H. *Berl. Bunsen Ges. Phys. Chem.* **90,** 1159 (1986).
116. Miller, A., Knoll, W. & Mohwald, H. *Phys. Rev. Lett.* **56,** 2633–2636 (1986).
117. Mullins, W. & Sekerka, R. K. *J. appl. Phys.* **34,** 323–329 (1963).
118. Moore, B. G., Knobler, C. M., Broseta, D. & Rondelez, F. *J. Chem. Soc. Faraday Trans.* II **82,** 1753–1761 (18987).
119. Marden, M. P. *Phys. Rev.* A **36,** 438–440 (1987).
120. Beenakker, C. W. J. *Phys. Rev.* A **37,** 1697–1702 (1988).
121. Stavans, J. & Glazier, J. A. *Phys. Rev. Lett.* **62,** 1318–1321 (1989).
122. Stine, K. J., Ranseo, S. A., Morre, B. G. Wise, J. A. & Knobler, C. M. *Phys. Rev.* A **41,** 6884–6892 (1989).
123. Berge, B., Simon, A. J. & Libchaber, A. *Phys. Rev.* A **41,** 6893–6900 (1990).
124. Lucassen, J., Akamatsu, S. & Rondelez, F. *J. Colloid Interface Sci.* (in the press).
125. Schwarz, H. A. *Gesammelte Mathematische Abhandlungen* Vol. 1 (Springer, Berlin, 1890).

ACKNOWLEDGEMENTS. The authors are especially indebted to T. Witten for very helpful comments. J.P. acknowledges extremely useful conversations with L. Leibler and A. Ajdari.

A REFINER'S APPROACH TO ASPHALT CHARACTERIZATION

Jean-Pascal Planche
Centre de Recherche
Elf Solaize,
BP 22, 69360
St Symphorien d'ozon,
France
Helen W. King,
Gayle N. King
Elf Asphalt, Inc.
Laboratory,
400 N 10th St,
Terre Haute,
IN, 47807, USA.

Asphalt, the earliest known adhesive, was first used in prehistoric times. Today, petroleum-based bitumen is the primary binder for roads and airport runways, roofing and applications as diverse as tennis court surfaces, running tracks, pipe coatings and automobile undersealants. The goal of Elf Aquitaine's bitumen research is to define the chemical, structural and physical properties that give the best long-term performance in these various applications.

Bitumen is not a pure product, but a colloidal mixture of many types of molecules with varying polarity, aromaticity and molecular weight. Its basic chemistry depends markedly on crude source and refining method. Bitumen characterization methods traditionally concentrate on three areas: chemical composition, colloidal structure and physical properties. Elf, one of the world's largest bitumen suppliers, recently launched a research study to characterize 100 different asphalts of various origins. From a refiner's perspective, this information is critical in preparing for the new specifications and requirements being defined by the European Community and the US Strategic Highway Research Program (SHRP). The latter is a five-year worldwide effort to redefine the techniques used to evaluate asphalt binders and mixes.

Conventional methods of testing the chemical composition of asphalt include elemental analysis (H, C, N, O, S, V, Ni and Fe) supplemented with liquid chromatography (eg SARA and Corbett) to separate asphaltene, paraffin, resin and aromatic fractions. More recently, a thin-layer chromatography technique, Iatroscan, has been developed to replace the large liquid chromatography columns with silica-coated glass rods.

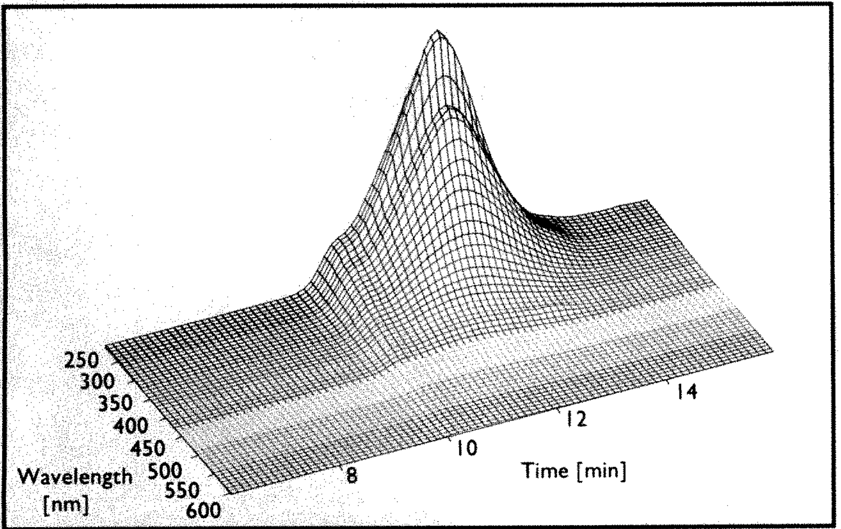
Automatic syringes control sample injection to improve precision, and flame ionization detects the separated fractions.

We have developed a high-performance gel-permeation chromatography (HP-GPC) technique incorporating a UV-visible photo-diode array detector[1]. By varying experimental conditions such as concentration, solvent, elution rate and temperature, many properties can be studied and the results compiled in only a few minutes.

By combining this information with some of the techniques listed below, the structure of the molecules in asphalt can be determined. Figure 1 is a three-dimensional HP-GPC curve showing the relative concentrations (Z-axis) of size groups (indicated by column retention time on the X-axis), as detected at varying wavelength (Y-axis).

The collaboration of international experts from industrial, academic, government and private research institutions has resulted in many significant contributions to asphalt characterization. For example, differential scanning calorimetry (DSC) methods have been developed to study quantitively the thermal behaviour of asphalts[2]. DSC is used to evaluate very small samples of bitumen over a temperature range of − 100 to + 220°C. The most common deficiency of asphalts used for road paving is its susceptibility to extremes of temperature (fracturing due to brittleness at low temperature or permanent deformation due to softness or fluid flow at high temperature). The thermal stability of a bitumen can be predicted by measuring the enthalpies of crystal formation during cooling, melting, solubilization, and vaporization of volatiles during heating. The glass transition temperature (T_g) can also be determined from DSC data. This parameter seems to predict the minimum temperature serviceability of an asphalt more accurately than does the currently used Fraass brittle temperature test[3]. DSC has also been combined with chemical composition analysis to determine the role of various components in thermal behaviour. By analysing the separate liquid chromotographic fractions individually, DSC can identify the contribution of saturated and aromatic molecular species to low-temperature instability (Figure 2). Aliphatic associations now referred to as "crystallizable fractions" were first identified using this technique, and later confirmed by phase contrast microscopy. The photomicrographs in figure 2 show the evolution of these microcrystals with temperature, and the corresponding enthalpy changes as measured

Figure 1: 3-Dimensional plot of HP-GPC data at multiple wavelengths.



by DSC associated with their formation and/or dissolution.

Work correlating the DSC results with rheological properties is continuing. The effect of cooling (and heating) rates on the structure of asphalt is also being studied. This dependence of structure on thermal history is a primary concern in developing accurate test methods for asphalt. The difference in heating and cooling curves (hysteresis) is evidence that steric hardening or other time dependent physical changes may play an important role in the ultimate performance of asphalt in a highway pavement.

To characterize the physical properties of asphalts, standard methods (such as vacuum capillary viscometry, penetration, ring and ball softening point, Fraass Point) have been supplemented by several new techniques. Of specific interest are new rheological methods and tension tests. Rotational viscometers and dynamic mechanical spectrophotometers are being used to study the effects of stress, frequency, temperature, and relaxation time on viscous and elastic components of asphalt complex viscosity. The aim is to develop a model that can describe the sol-gel transitions of asphalt, to predict the conditions at which the material undergoes these transitions, to relate this behaviour to thermal effects detected by DSC, and ultimately to find correlations with chemical composition. Tests are run at temperatures of −150 to +350°C, sinusoidal frequencies of 10^{-3} to 20 Hz, relaxation times of 20 ms to 10s, and under air or nitrogen atmospheres. Cone and plate, plane-plane, concentric cylinder geometrics are being studied.

Using this data, improved products are being engineered. In the 1970s, Styrelf, a process for modifying asphalt with styrene-butadiene block copolymers, was developed and patented in a cooperative research effort between Elf and the French Laboratoire Central des Ponts et Chaussees in Paris. Styrelf chemically crosslinks a styrene-butadiene block copolymer to the asphalt in-situ. In order to characterize the new modified asphalt, several testing procedures have been developed.

These include adapting sophisticated rubber tension test equipment to research the stress-strain properties of polymer-modified asphalt, and developing new, cheaper equipment for quality control laboratories. The force ductility test uses an inexpensive adapter to measure the force required to stretch a moulded specimen in a standard asphalt ductilometer. This procedure was recently approved for preliminary publication by the US standards body ASTM.

Perhaps the most critical property in asphalt applications is its compatibility with polymers. The relationship of microstructure to physical properties is being studied by optical reflective fluorescence microscopy and transmission electron microscopy. In the first method, polymer-modified asphalt specimens are frozen, fractured, and bombarded with blue light. The oils swelling the polymer phase fluoresce yellow light, while the bitumen remains dark.

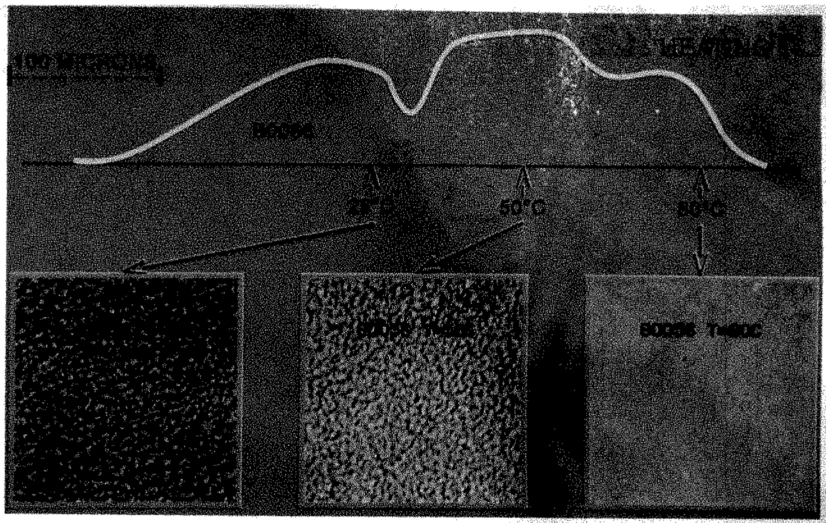


Figure 2: Correlation between DSC and thermomicroscopy.

The microstructure, as characterized by the composition, relative concentrations and particle dimensions of continuous and dispersed phases, shows significant variation attributable to the asphalt. Photomicrographs of an asphalt-polymer system clearly distinguish incompatible mixtures from homogeneous, swollen blends.

In the transmission method, thin wafers are prepared by cryofracture and stained with OsO_4. Correlations have been established between microstructure and physical properties such as tensile strength at elongation.

On the practical side, formulations and processes for cationic, anionic and nonionic emulsions for many different paving techniques are being optimized continually. Of particular note is a Styrelf emulsion specially designed for seal coating during weather conditions normally considered too cold or severe for conventional surface treatments. An important area of study is the effect of bitumen binder in the finished asphalt-aggregate mix. Research projects in France, Germany and the United States have closely monitored such parameters as mixture stability, rutting resistance, fatigue resistance, low temperature cracking potential and resistance to moisture damage.

To ensure consistent product quality, new methods are being developed for manufacturing modified apshalts and emulsions. New processes, more efficient mixing and milling units, in-line liquid mass flow meters, on-line monitoring systems, new safety equipment, and energy saving options are some of the topics under study.

Powerful new analytical techniques for asphalt characterization are now being investigated by Elf researchers and affiliated laboratories. Supercritical fluid chromatography (SFC) is a new method for fractionating heavy petroleum residues into basic chemical families. Fourier-transform infrared spectroscopy (FTIR) is being used to characterize the organic molecules absorbed most strongly on a mineral surface. Electron paramagnetic resonance (EPR) can identify free radicals and unpaired electrons in metal ions, tracking their catalytic effect on asphalt oxidation.

Although asphalt has a very long history, current research is about to turn it into a high tech product.

REFERENCES

1) PRIBATIC J., EMMELIN M., KING G.N., Use of a multi-wavelength UV-vis detector with HP-GPC to give a 3-D view of bituminous materials. 4th Eurobitume Symposium 1, 151 (1989).
2) CLAUDY P., LETOFFE J.M., KING G.N., PLANCHE J.P., BRULE B., Characterization of paving asphalts by differential scanning calorimetry. Fuel Science and Technology International 9, 1, p71 (1991).
3) BRULE B., PLANCHE J.P., KING G.N., CLAUDY P., LETOFFE J.M., Relationships between characterization of asphalt cements by differential scanning calorimetry and their physical properties. Preprints ACS Symposia 35, 3, p330 (1990).

ADVANCES IN POLYMERIC PIEZOELECTRIC TRANSDUCERS

Richard A Ferren
Atochem Sensors Inc
950 Forge Avenue
Norristown, PA 19403
USA

Multi-element sensor on a single sheet of piezoelectric film

Transducers can now be manufactured from polymers - opening up vast new application areas because of the ease with which polymers such as vinylidene fluoride can be injection moulded, solution cast, extruded and compression moulded into a wide variety of shapes and sizes. Large and continuous areas of flat film can be manufactured by conventional film extrusion and stretching equipment, while small parts can be made by compression and injection moulding. Multiple sensors can be fabricated from a single piece of film by depositing multiple electrodes on the film.

Polyvinylidene fluoride can be extruded into rolls of film and into flat sheets. Film rolls can be thousands of metres long, with thickness varying from 25 to 500 micrometres thick. The films are first highly stretched to convert the polymer into a polarizable form, which decreases the leaf thickness to as little as a few micrometres and increases the film area; they are then polarised to give them ferroelectric, pyroelectric or piezoelectric characteristics. Electrodes are then deposited on both surfaces of the film for connection to electronic lead lines or contacts.

The transduction can involve conversion of electrical energy to mechanical, mechanical to electrical, or thermal to electrical energy.

A typical application for large area film transducers is as traffic sensors. Long lengths of film are either buried or laid on roads to sense traffic movements, vehicle speed or weight. As a vehicle passes over a sensor, a charge redistribution occurs in the film causing the current to flow; the electronics in the sensor pick up the signal and make the appropriate response. Electrodes are normally made from a silver ink that can withstand the stresses of passing traffic. Similar pressure-sensitive large area sensors are used in security applications.

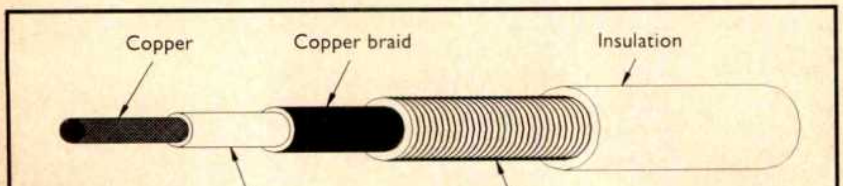


Figure 1.
Piezoelectric wire

Large area sensors may also be fabricated from wire and cable components. The simplest such sensor consists of a core wire with a vinylidene fluoride copolymer coating extruded over it, a conductor then coating the fluorocarbon polymer and an outer coating of another polymer such as polyvinyl chloride. The polarisation process is carried out continuously. Vinylidene fluoride does not have to be oriented to be polarizable, since the copolymer crystallises directly into a form that is readily polarized to be piezoelectric (Figure 1).

SMALL-AREA SENSORS

A piezoelectric plastic film is insulating. Arrays of small senors can be created by inducing metallization of discrete areas with surrounding areas remaining insulating. The back side of the film either has a matching pattern of metallized areas or is completely metallized to form the electrical ground for all its elements.

One of the great advantages of piezoelectric film is that it is very robust. It can be bent, punched, drilled and cut without fracture. Because the film is an excellent insulator metallized areas can be very close together and yet electrically isolated from each other. Electrical connections to the film metallization may be made with conductive adhesives, low melting solders, wire bonding, and mechanical contacts. An example of a mechanical contact is to insert a grommet through the hole, on top of which an eyelet has been placed. The eyelet in turn is attached to the lead wire (Figure 2). Being unmetallized at the site of attachment, the opposite side of the film does not cause electrical shorting. Figure 2 shows a transducer element with metallization on both film surfaces and the appropriate electrical connections attached.

VACUUM METALLIZED AND SCREEN PRINTED SENSORS

Complex patterns can be made on piezoelectric film by making a positive metal mask of the pattern and metallizing through the mask onto the film. Details of the vacuum metallization process are important in assuring good dimensional tolerances of patterns. Piezofilm made from polyvinylidene fluoride homopolymer has to be oriented to transform the crystalline state of the polymer from a non-polar to a polar form. A consequence of this orientation is to produce a film susceptible to shrinkage and loss of orientation when the film is thermally treated. Vacuum metallization by sputtering is the best way of depositing metal on film because this is a cold process which avoids shrinkage of the film during metallization.

Conductive inks can be used to print piezo films with patterns with 200 micrometre lines and spaces. This is easier and cheaper than batch metallization. Cost differentials between continuous vacuum metallization and continuous printing depend on volume. The

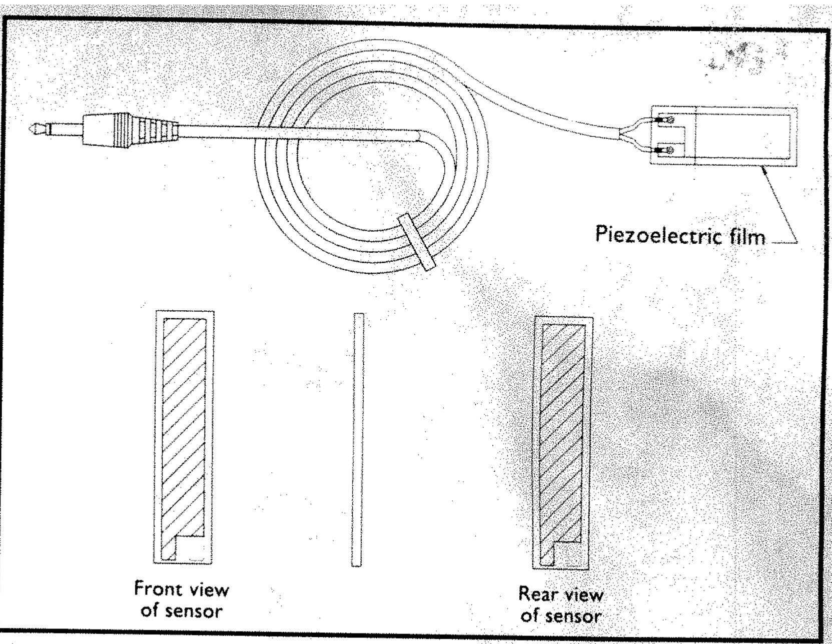


Figure 2.
Piezoelectric sensor

vacuum method is only economical at higher volumes because of the amount of time it takes to evacuate the vacuum chamber.

Metallization thickness of screen printed patterns is about 12 micrometres whereas it is only 0.1 micrometre thick on vacuum metallized films. The cost of the silver inks is therefore a significant cost factor. The printing also takes longer than for vacuum metallization because of the need for solvent evaporation, which is also an environmental concern.

ETCHED PATTERNS

Conventional pattern etching on piezo-film can be done with standard dry and wet film resist technology, and by newer methods, including laser ablation and electrophoresis. Other processes such as plasma dry processing, X ray lithography, gas processing and direct etch, are possible but more development work is needed before these methods can be used commercially.

Vinylidene fluoride copolymer films can also be formed by casting on continuous metallic or polymeric surfaces.

With appropriate controls the surfaces of these copolymer films can be very uniform and free from defects. The pattern resolution of these copolymer films is superior to homopolymer film but because copolymers dissolve in many common solvents this limits the choice of liquid resists.

Films can be made in thicknesses of less than one micrometre by casting. In addition to piezoelectric and pyroelectric applications involving small thicknesses and intricate patterns, applications based on the ferroelectric property of the copolymer are also possible. The electric field needed to switch the polarization of the copolymer is large, but using extremely thin film can reduce the required voltages to practical levels.

LASER ETCHED SENSORS

Polyvinylidene fluoride film can be cut by lasers such as the CO_2 laser, but this leaves a carbonaceous residue which may be a problem if it coats metallized areas on the opposing film. Siemens, the German electronics company, Atochem and others have found it is possible to pattern piezo-film with excimer lasers. These ultraviolet lasers are particularly good for etching patterns on polyvinylidene fluoride film which is ultraviolet transparent. Polymer ablation is a problem only when high-power lasers are used. In fact patterns in perfect registration are obtained when metallized film is ablated with an excimer laser such as the argon fluoride laser. Because the film is transparent to the laser, metallization is ablated front and back. At low laser power, the back side metallization is more uniformly removed than the front. Laser patterning at high speed with good resolution is now possible.

MULTILAYER TRANSDUCERS

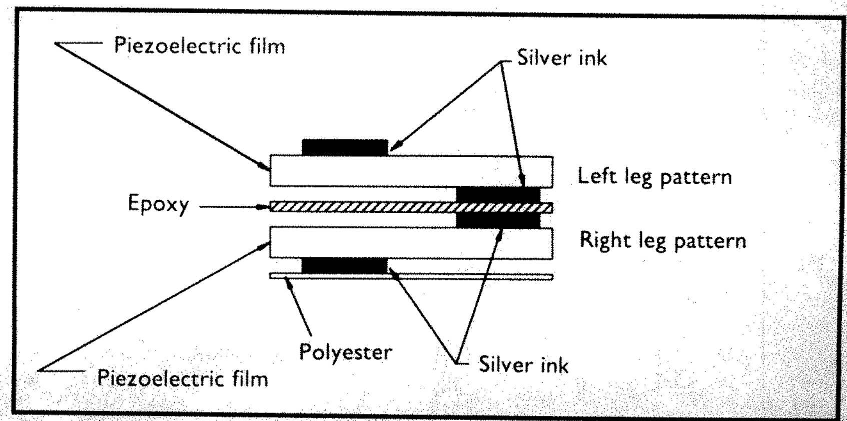
Piezoelectric films stacked with identical polarity are equivalent to a single film of the stack thickness. If two films are stacked with the polarities reversed one film stretches, while the other contracts, causing bending. If a parallel electrical signal is applied to a stack with identical film polarization bending also occurs. These arrangements are called biomorphs (Figure 3). Multilayer stacks of one hundred or more have been made to multiply the energy that can be obtained from the films for spark generation.

The films can be made self shielding by folding them, so that the signal is restricted to the inner surface, the outer surface is the ground. Shielding is very important where noise problems are encountered which is often the case since piezo-electric films are excellent pick-ups.

SENSORS FORMED BY MOULDING

Vinylidene fluoride copolymers can be moulded by conventional thermoplastic moulding procedures, and sensors have been made using most of these procedures. The resin is injection moulded to form shapes such as half sphere domes and tubes which are then polarized and metallized to form sensors. Wire coated with the copolymer can be polarized either during or after the extrusion process. Compression moulding is used in the same way. Polymer film sensors may be fabricated in the same configuration as conventional sensors, but these films also offer the promise of building sensors that previously had not been either feasible or practical. An added incentive to the development of these sensors is the possibility of using high volume production equipment and methods for their manufacture.

Figure 3.
Bimorph made from two sensors



POLYMER DESIGN FOR HIGH PERFORMANCE MATERIALS: LIQUID CRYSTAL POLYMERS

Didier Judas,
Yves Germain
Atochem
Cerdato BP 19
27470 Serquigny
France
Claudine Biver,
Sylvie Girault,
Christiane Taupin
Atochem-CAL
95 rue Danton
92300 Levallois
France

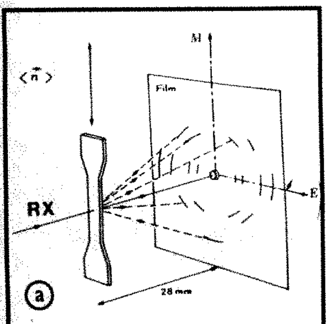


Figure 1: (1.a.) Photographic set up; ‹n› is the axis of orientation. (1.b.) Nematic state of small rod-like molecules and (1.c.) corresponding diffracted diagram. (1.d.) Nematic state of main chain copolymer and (1.e.) corresponding diffracted diagram. The relative sizes of rod-like molecules and comonomers A and B are realistic.

Low-molecular-weight liquid-crystal compounds have been known and studied for nearly 100 years. Study of the effect of segmental rigidity in polymers led Flory[1] in 1956 to formulate criteria for the existence of an anisotropic phase in polymer solutions: these also apply to thermotropic polymers. The first published description of a thermotropic polymer based on main chain conformation appeared in 1975, about 20 years after the discovery of liquid crystal polymer solutions[2]. Three papers[1-3], two from industrial companies, were concerned with studies of polyesters. Side chain thermotropic polymers were discovered at the same time.

Industrial interest in main chain liquid-crystal polymers was stimulated by the general properties of systems that exhibit a nematic phase. Among the properties which affect the processing of fibres or other morphologies is the response of the nematic phase to shear. Alignment of the nematic domains parallel to flow induces remarkably low apparent viscosities. Under such conditions the product becomes highly oriented, as in drawn fibres. The mechanical properties of such undrawn products thus greatly surpass those of conventional fibres and films.

The chemical units used for thermotropic polymer formation have been described by Lenz[6] and McIntyre[7]. The melting temperatures of many homopolyesters are too high (around 600°C) to prepare and observe a stable nematic melt. To obtain a liquid crystal phase below the polymer's decomposition temperature, the melting point of the polymers is reduced by using comonomers to disrupt the order in the chain.

Various comonomers can be used:

- Differently sized aromatic units[8-12] (melting points in the range 265 - 400°C)
- Nonlinear aromatic units introduced into the linear rigid-rod structure. These reduce the melting point of the polymer but also decrease the thermal stability of the liquid-crystal phase[13].
- Lateral substitutes on the aromatic ring[14-16]. The size is very important (methyl, chlorinated and phenyl substitutes are particularly efficient).
- Inserting flexible spacers into the linear rigid-rod structure. Jackson and Kuhfuss have published several papers and patents describing the properties of this kind of polymer[5-17].

All of these structural modifications reduce melting temperatures, allowing the polymer to be injection moulded.

THE STRUCTURE OF A MAIN-CHAIN NEMATIC COPOLYMER

The nematic state is represented in Figure 1b. The molecules are parallel to each other, but there is no correlation between their positions. This model accounts for the fluid and anisotropic properties of the liquid-crystal state. The internal organization of such an oriented system can be seen with X-ray diffraction (Figure 1a).

In the diffraction pattern of a conventional small-molecule nematic phase (Figure 1c), a broad spot on the equatorial axis is associated with the lateral intermolecular distance d_I, and on the meridional axis, such a spot characterizes the repeat distance d_L along the molecules. This peak, much nearer to the incident beam at the centre of the pattern, corresponds to a large distance of around 20 - 30Å.

The reflection spot is crescent-shaped because of the angular spread in molecular alignment. (This implies that the order parameter, which defines the degree of alignment, is less than one.)

In the diffraction pattern for the nematic state of a liquid crystal polymer (Figure 1d) some features remain on the plate (Figure 1e), namely the equatorial broad line spot associated with the mean distance between the chains and the crescent shape of this diffraction spot, reflecting the angular spread of the nematic axes.

On the other hand, the meridional axis shows two important differences. Several lines appear (as opposed to a single one in the case of non-polymeric molecules). These aperiodic lines correspond to much smaller distances (a few ångströms), typically the length of the comonomers.

A detailed analysis of the diffraction lines along the meridional direction was made by Blackwell et al[19-21]. These referred to wholly aromatic copolyesters prepared from 4-hydrobenzoic acid (HBA) and 2-hydroxy-6-naphthoic acid (HNA) with various ratios of the two comonomers. The authors observed that the positions of the meridional lines vary with the comonomer ratio.

It has been shown[20] that the positions of these maxima correspond to chains that have a completely random sequence of monomers. A first approximation models the monomers as points separated by the appropriate monomer length. A more elaborate calculation taking the internal structure of the monomers into account, shows good agreement not only for the spot positions but also between their observed and calculated intensities.

Blackwell et al also determined the flexibility and persistence length (of the order of several tens of Å) of the macromolecular chain[20].

Our studies with different comonomers confirm these findings on the randomness of copolymerization and flexibility of chains. Another important property of liquid-crystal polymers is their ability to be oriented during process condition.

This produces improvements in some mechanical properties, in particular a higher modulus in the extension direction. Figure 2 follows the evolution of the X-ray diffraction pattern during the processing of an injected test piece, a film and a fibre. The angular spread of the nematic spot decreases, revealing an increase in the degree of orientation up to an almost perfect orientation for the high-modulus fibre. This orientation effect is much more marked in liquid-crystal polymers than other polymers. It is important in manufactured materials which exhibit strongly anisotropic properties.

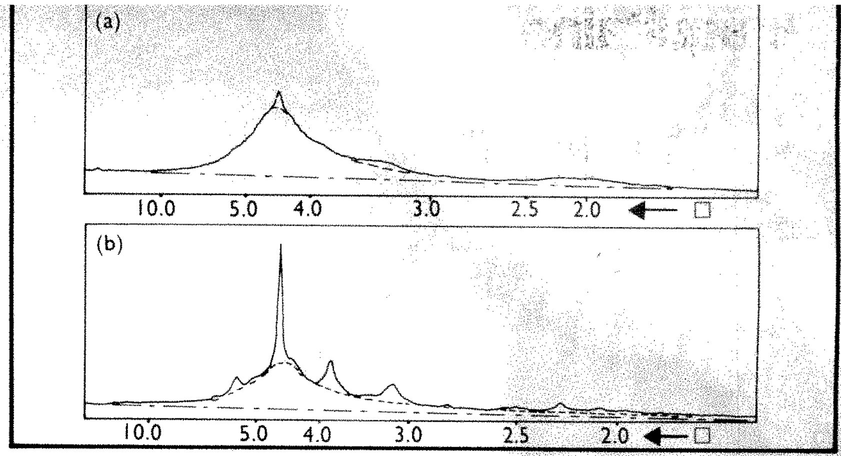


Figure 2: The spectrum recorded along the equatorial axis presents, for sample b, several Bragg peaks which are superimposed on the nematic broad line located at 4.5 Å. Crystallinity of b is larger than that of sample a.

COEXISTENCE OF NEMATIC AND CRYSTALLINE PHASES IN LCP

Thermotropic polymers are usually processed at temperatures corresponding to the liquid-crystal state and then cooled down to room temperature. Cooling causes some crystallization, as seen by recording the scattered intensity along the equatorial direction (Figure 3). Superimposed on the nematic broad line located at 4.5Å are several Bragg peaks. Their relative intensity, ie the amount of crystalline phase in the sample, depends on the nature and the concentration of the comonomers and the thermal history of the sample: annealing increases the crystallinity.

Semicrystalline polymers exhibit interesting mechanical properties due to the composite structure in which crystallites are embedded in an amorphous matrix. The role of this crystalline fraction in the properties of liquid-crystal polymers needs to be investigated[22] and controlled to improve the quality of finished products.

One interpretation of the solid-state formulation process in liquid crystal polymers was given by Butzbach et al[23]. Their view has provided the backdrop to Elf's approach to solid-state analysis.

The nematic phase is characterized by parallel orientation of the molecules with a large macroscopic order parameter. Cooling the nematic phase down to a lower temperature triggers the formation of a three-dimensional lattice perpendicular to the direction of the chain axes. These structures give rise to the Bragg peaks obtained in the X-ray patterns of Figure 3. This transition supposes that stable crystallites link together to form rigid chains. In that sense the crystals play an analogous role to those in conventional polymers, and have a major role in determining mechanical properties. However there are distinctions in the nature of the crystals in liquid-crystal polymers, as pointed out by Blundell.[24]

Principal among these is the low surface energy of the crystallites, which gives rise to the stability of very small crystals as well as to their ease of nucleation. These features are a necessary precondition for the formation of solid crystals in random coploymers. Only a small percentage of crystallizable units can be involved in the formation of the crystallites since the unit sequences in the chains are very short. Factors that influence the distribution of comonomers along the chain such as their composition and chemical nature, will influence crystal formation.

A computational study of the degree of solid state order which can be attained by varying these parameters has been carried out using the model of chain-like systems as proposed by Windle et al[25] and Tomka[26]

The model is based on an algorithm for generating random chains taking into account two major constraints: the mean composition of comonomers over the whole set of generated chains, and the rules for monomer chemical structure, for example, if B denotes a diacid molecule, B-B segments are prohibited. Around 1,000 chains are generated. The degree of copolymerization is arbitrarily assigned as 50.

The second step involves matching identical units in adjacent chains. The groups of matching segments are defined as the crystalline part of the copolymer. Some units cannot crystallize either because of their surroundings or because of their inherent structure. These physical data are of major importance in building the model, and require studies of the homopolymers of the specific comonomers.

Shifts of chains along their axis to improve the match are forbidden. This corresponds to a quenched condition. The amount of crystals is computed by enumerating the number of matched units along a chain and on adjacent chains.

Figure 3 shows the result for a four-comonomer liquid crystal polymer. One of the monomers does not crystallize. Chains appear as vertical red lines while blue represents crystallites. The amount of crystallites is found to be about 40% which is close to the experimental value obtained under quenched conditions.

Work is continuing at Elf Aquitaine to optimize the mechanical properties of nematic copolymers as a function of their chemical structure, yielding improved final products.

Figure 3

REFERENCES

1) P.J. FLORY, Proc. Roy Soc. A 234, 73 (1956).
2) C. ROBINSON, Trans. Faraday Soc. 52, 571 (1956).
3) A. ROVIELLO, A. SIRIGU, J. Polym. Sci. Polym. Latters Ed. **13**, 455 (1975).
4) Du PONT Belg. P., 828-935 (10.05.74-USA).
5) W.J. JACKSON, H.F. KUHFUSS, J. Polym. Sci. Polym. Chem. Ed. **14**, 2093 (1976).
6) R.W. LENZ, Recent Advanced in Liquid Crystalline Polymers, 3-13 (Elsevier Applied Science Publishers) (1986).
7) M.G. DOBB, J.E. McINTYRE, Advanced in Polymer Science **60, 61**, 63-97 (1984).
8) W.J. JACKSON, J.C. MORRIS, Brit. Patent 2, 001, 404 (08.08.77).
9) S. COTTIS, G. ECONOMY, J. WOHRER, Belg. Patent 1, 479, 513 (25.01.75).
10) G.W. CALUNDANN, Belg. Patent 1, 585, 511 (13.05.76).
11) G.W. CALUNDANN, Brit. Patent 2, 006, 242 (20.10.77).
12) G.W. CALUNDANN, US Patent 4, 184, 996 (15.02.78).
13) R.W. LENZ and J.I. JIN, Macromolecules **14**, 1405 (1981).
14) I. GOODMAN, J.E. McINTYRE, J.W. STIMPSON, Belg. Patent 989, 552, (19.02.62).
15) I. GOODMAN, J.E. McINTYRE, D.A. ALDRED, Belg. Patent 993, 272, (22.05.62).
16) J.R. SCHAEFGEN, Belg. Patent 1, 507, 207 (10.05.74).
17) W.J. JACKSON, H.F. KUHFUSS, Belg. Patent 1, 435, 021 (28.09.72).
18) F. HARDOUIN, A.M. LEVELUT, J. Physique **41** (1980), 41-46.
19) R.A. CHIVERS, J. BLACKWELL and G.A. GUTIERREZ, Polymer **25** (1984), 435-440.
20) A. BISWAS and J. BLACKWELL, Macromolecules **20** (1987), 2997-3002.
21) S.K. HONG and J. BLACKWELL, Polymer **30** (1989), 780-785.
22). P.J. BROWN, I. KARACAN, J. LIU, J.E. McINTYRE, A.H. MILBURN and J.G. TOMKA, Polymer International **24** (1991), 23-32.
23) G.D. BUTZBACH, J.H. WENDORFF and H.J. ZIMMERMANN, Polymer **27** (1986), 1337-1343.
24) D.J. BLUNDELL, Polymer **23** (1982), 359-363.
25). S. HANNA and A.H. WINDLE, Polymer **29** (1988), 207-223.
26) V. KALYSAS, Z.G. LI, J.E. McINTYRE, A.H. MILBURN and J.G. TOMKA, PPS proceedings (1990) Nice, France.

CHEMICAL SYNTHESIS OF GLYCOSAMINOGLYCANS: NEW APPROACHES TO ANTITHROMBOTIC DRUGS

Maurice Petitou,
Jean-Claude Lormeau,
Jean Choay
Sanofi Recherche,
9 rue du Président
Salvador Allende,
94256 Gentilly France

Sanofi, Elf Aquitaine's pharmaceutical subsidiary is a world leader in antithrombotic drugs. This article traces the development of Heparin-based antithrombotic drugs and describes the company's progress in producing synthetic substitutes.

Glycosaminoglycans (heparin, heparan sulphate, chondroitin sulphate, dermatan sulphate, hyaluronic acid and keratan sulphate) are among the most intricate structures in living organisms[1]. Until 10 years ago study of these anionic polysaccharides was impeded by a lack of suitable research tools but as a result of new techniques[2-4] the physiochemical and biological properties of glycosaminoglycans can now be studied in detail. Most recently the action of dermatan sulphate[5] and heparan sulphate[6] has been investigated. But the most famous example of glycosaminoglycan activity remains the action of heparin in blood clotting.

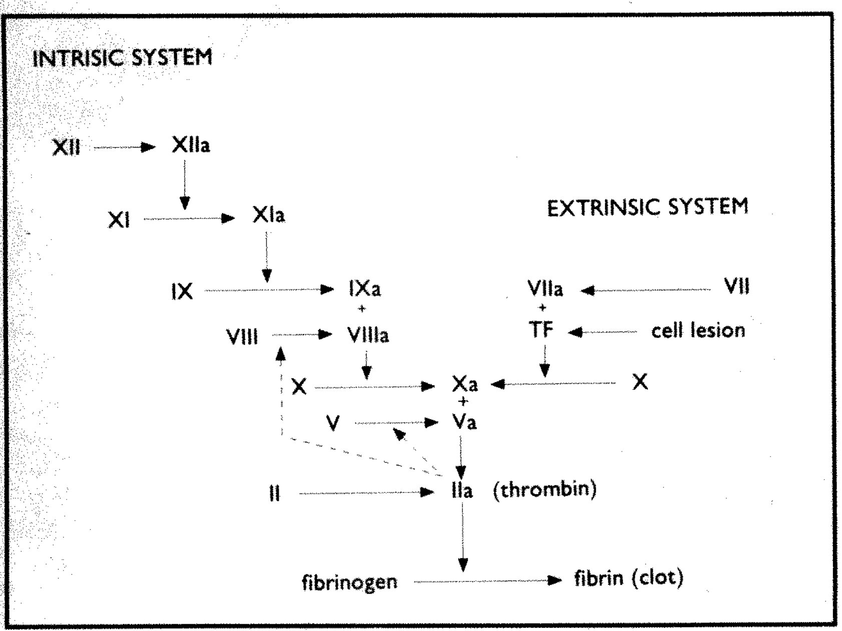


Figure 1: **Heparin and the blood coagulation system.** The coagulation of blood is a complex process involving several enzymes (factors and cofactors of coagulation) present in plasma as their precursors (zymogens). Activation of these pro-enzymes occurs in cascade and can be initiated by cell thromboplastin (tissue factor) via the extrinsic pathway. It can also be triggered through the intrinsic pathway by contact of blood with subendothelial structures exposed by damaged blood vessels or with foreign wettable surfaces. The two pathways converge at the level of factor X activation. It was also recently confirmed that factor IX can be activated by factor VII and tissue factor[39]. The dotted lines indicate the feedback activation by thrombin: the first traces of thrombin activate cofactors VIII and V which in turn accelerate thrombin generation. An inbalance in the system leads to a prohaemorragic or to a prothrombotic state. Physiological inhibitors like antithrombin III are supposed to damp blood coagulation. When it is complexed to heparin it can inhibit all activated blood coagulation factors except factor VIIa. Inhibition of factor Xa and factor XIIa can be promoted by any heparin chain, whatever its size, bearing the antithrombin III binding site, but inhibition of factor IXa, XIa and thrombin requires not only binding to antithrombin III but also a chain length of 16-18 monosaccharides at least, to attract the protease itself. This explains the different anti-factor Xa/anti-thrombin ratios observed between standard heparin (ratio ≈ 1) and low-molecular-weight heparin (ratio > 1).

Heparin was discovered in 1916, but it was not until 20 years later that it was first used for preventing venous thrombosis. Since then there have been major improvements in its purity and mode of administration, and the drug is now widely used for the prevention and treatment of cardiovascular disorders, particularly thrombosis[7].

The mechanism by which heparin works on blood clotting remained unknown until the heparin cofactor in plasma antithrombin III (AT III)[8,9] was identified, and the active species in heparin preparations fractionated. AT III, a 432 amino acid protein (in humans) is a natural inhibitor of several blood coagulation factors (see Figure 1), and its potency is dramatically increased in the presence of heparin (x 1,200, x 2,000 and x 10,000 with respect to factor Xa, thrombin and factor IXa inhibition respectively[10]). Following the identification of AT III, it was shown that not all the molecules in a heparin preparation can bind and activate this compound[11-13]. In fact heparin preparations can be divided into high-affinity and low-affinity fractions, with only the former having marked anticoagulant activity.

At the same time, gel filtration chromatography experiments indicated that this anticoagulant activity depends on molecular weight (see legend to Figure 1) and that shorter chains could promote factor Xa inhibition preferentially[11,14].

Understanding the action of heparin prompted the development of low-molecular-weight heparins with improved antithrombotic activity.

In parallel with these discoveries new theories were proposed to describe the pharmacological action of heparin and the influence of factor Xa and thrombin inhibition in the prevention of thrombosis[15]. It was concluded that heparin preparations with an increased ratio of anti-factor Xa to anti-factor IIa activity are more effective antithrombotic agents and this was confirmed by clinical trial[16,17]. The improved pharmacokinetic properties of low-molecular-weight heparins mean that patients need only one injection per day, rather than the two needed with classical heparin.

A SHORT SPECIFIC SEQUENCE IN HEPARIN ACTIVATES THE ANTICOAGULANT PROTEIN ANTITHROMBIN III

To further investigate the antithrombotic potency of products with improved anti-factor Xa activity, the shortest fragments still able to catalyse the AT III - factor Xa reaction were isolated[18,19] and their structures elucidated (see Figure 2 and legend). Such fragments were completely devoid of activity in the thrombin inhibition assay even though their antithrombotic activity was demonstrated in animal models. The fragments were independently reported to correspond to the unique sequence responsible for recognition and binding of heparin by AT III[20].

At this stage of the research the AT III mediated anticoagulant activity was explained as follows[21]: the specific recognition sequence (pentasaccharide of Figure 2) binds to AT III and induces a conformational change which is sufficient to dramatically increase the kinetics of interaction with factor Xa. On the other hand, efficient thrombin inhibition requires longer fragments, able to accommodate not only the AT III molecule (again through the AT III binding site) but also thrombin itself. In this case, heparin acts as a template which favours the AT III - thrombin interaction.

SYNTHETIC OLIGOSACCHARIDES - ANTITHROMBOTICS OF THE FUTURE

The discovery that short oligosaccharide fragments are able to induce anti-factor Xa and thereby antithrombotic activity opened up the possibility of obtaining antithrombotic derivatives by chemical synthesis. Heparin and low-molecular-weight heparin are heterogeneous mixtures of polysaccharide fragments with multiple sites of action - a product designed to act at a single site would have more pharmacological specificity. Structural studies (see legend to Figure 2) showed that the target oligosaccharide was the pentasaccharide sequence DEFGH. This triggered a research effort in chemical synthesis of glycosaminoglycan fragments[22,23] and the first successful efforts to do this were reported in 1983.

The preparation of DEFGH was made difficult by the presence on specific positions of the target molecule of iduronic acid (the chemistry of which was unknown), glucuronic acid, N-acetyl-glucosamine, N-sulphate-glucosamine and various sulphate esters and hydroxyl groups. A synthesis route starting from monosaccharides was developed for the non-N-acetylated derivative (compound 1, table 1), producing the final product in 75 steps[25,26](see Figure 3). This bound to AT III with the same affinity as heparin and activated AT III against factor Xa selectively. Other preparations of the same molecule have since been reported[27,28] using similar strategies, and the slightly more complex N-acetylated sequence has also recently been obtained[29].

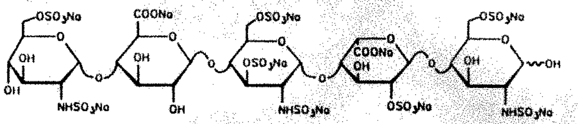
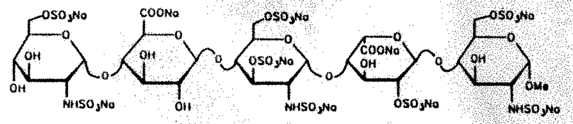
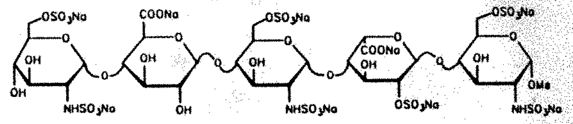
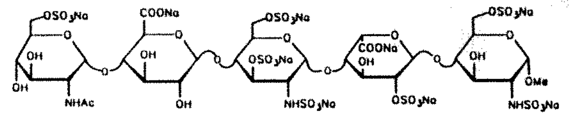
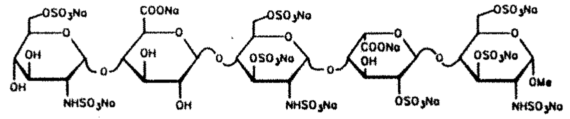
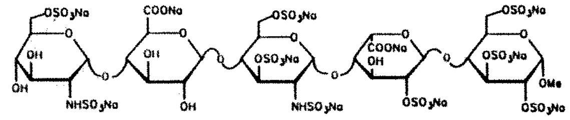
		anti-Xa (units/mg)	K_D (nM)
1		600	50
2		600	50
3		1<	$>2.10^6$
4		320	80
5		1300	5
6		1300	3

Table 1: **Biological properties of some synthetic pentasaccharides. The dissociation constant for AT III binding was determined by fluorescence spectroscopy as described[37]. Anti-factor Xa activity was measured using pure factor Xa, pure AT III and a chromogenic substrate, as already reported[38]. Comparison of 2 and 3 demonstrates the key role of the 3-O-sulphated glucosamine unit. Most remarkable is the activity of compound 5 which contains two 3-O-sulphated glucosamine units and the activity of 6 in which one N-sulphate has been replaced by an O-sulphate group.**

Synthetic oligosaccharides provide the ideal tools for investigating the structure/activity relationships in heparin binding to AT III, and the biological activities[24] of several other compounds are shown in table 1.

The two tetrasaccharides lacking either of the glucosamine end units were also obtained and shown to have very little affinity for AT III, indicating that a pentasaccharide is required. This sequence contains a disaccharide (EF) made up of glucuronic acid and 3,6-di-O-sulphate-N-sulphate glucosamine, two monosaccharides that occur only in minute amounts in heparin but which are characteristic of the AT III binding site. The role of the

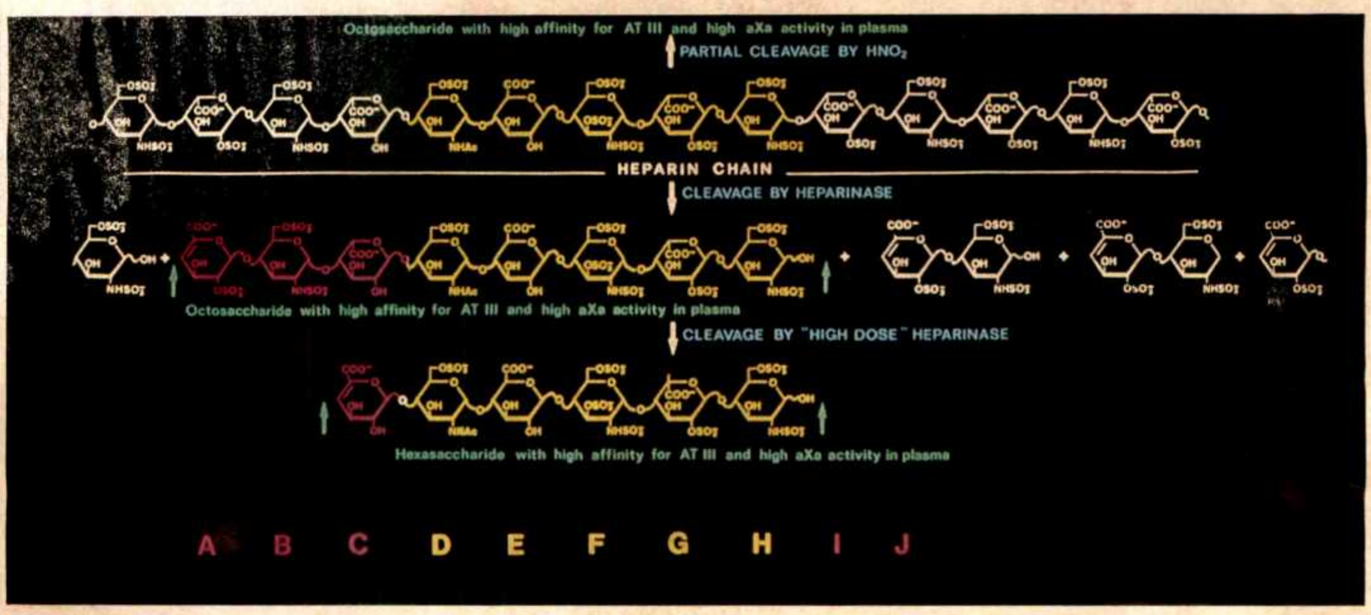

Octosaccharide with high affinity for AT III and high aXa activity in plasma
PARTIAL CLEAVAGE BY HNO₂
HEPARIN CHAIN
CLEAVAGE BY HEPARINASE
Octosaccharide with high affinity for AT III and high aXa activity in plasma
CLEAVAGE BY "HIGH DOSE" HEPARINASE
Hexasaccharide with high affinity for AT III and high aXa activity in plasma
A
B
C
D
E
F
G
H
I
J

Figure 2: **Heparin and heparin-derived oligosaccharides.** Standard heparin is a mixture of polysaccharide chains consisting of a repeated basic disaccharide sequence made up of a uronic acid and a glucosamine which are 1→4 linked. Depending on the length of the chain, there are between 10 and 30 disaccharides. 2-O-sulphate-α-L-iduronic acid (G,I) and 6-O-sulphate-N-sulphate-α-D-glucosamine (B,H,J) are the predominant monosaccharides and the repetition of trisulphated disaccharides like GH and IJ constitute the so-called "regular sequences" which account for the major part of the structure. Other monosaccharide residues like α-L-iduronic acid (C) 6-O-sulphate-N-acetyl-α-D-glucosamine (D) β-D-glucuronic acid (E) an 3,6-di-O-sulphate-N-sulphate-α-D-glucosamine (F) are much less common and occur in "irregular regions". The latter are apparently related to the biological activity as indicated by the critical role played by units E and F in the binding of heparin to its "receptor" antithrombin III (see text)- 2-O-sulphated-β-D-glucuronic acid is present in some heparin preparations. A certain number of glucosamine units (less than 20% in general) are not sulphated at position 6. Altogether 10 different monosaccharides (4 uronic acids and 6 glucosamines) appear in heparin, making the overall structure a very complex one[1]. The biological significance of this complexity is not yet understood. A heparin chain can split up either chemically or enzymatically into fragments of various sizes. In case of heparinase enzymatic cleavage, α-βunsaturated-uronic acid residues constitute the non-reducing end of the new oligosaccharides. Partial degradation of a heparin chain containing the antithrombin III binding sequence leaves fragments that may contain this sequence as well as fragments that do not. The former can be sorted out by affinity chromatography on agarose bound antithrombin III. Further fractionation by gel filtration yields octa-, deca-, dodecasaccharides etc. The structure of the shortest fragments (octasaccharides) was determined by chemical and NMR methods[19]. When submitted to a high dose of enzyme the octasaccharide was transformed into the hexasaccharide of the lower line. On the basis that the conformation of the reducing end unit in this hexasaccharide was altered by loss of the asymmetry at C-5, it was hypothesized that the pentasaccharide D-H could represent the true binding sequence of heparin to antithrombin III[18].

REFERENCES:

1) RODÉN L., in The Biochemistry of Glycoproteins and Proteoglycans, W.J. Lennarz Ed., Plenum Press, New York 1980, pp. 267-371.
2) FRANSSON L.Å., HAVSMARK B. and SILVERBERG I., Biochem. J. **269,** 381-388 (1990).
3) LINHARDT R.J., RICE K.G., KIM Y.S., LOHSE D.L., WANG H.M. and LOGANATHAN D., Biochem. J. **254,** 781-787 (1988).
4) BOSSENNEC V., PETITOU M. and PERLY B., Biochem. J. **267,** 625-630 (1990).
5) MAIMONE M.M. and TOLLEFSEN D.M., J.Biol. Chem. **265,** 18263-18271 (1990).
6) FEDARKO N.S., ISHIHARA M. and CONRAD H.E., J. Cell. Physiol. **139,** 287-294 (1989).
7) For general recent reviews on Heparin, see: Heparin, LANE D.A. and LINDAHL U. Eds, Edward Arnold, London, 1989; and Heparin and Related Polysaccharides, Ann. NY Acad. Sci. **556** (1989).
8) ABILDGAARD U., Scand. J. Clin. Lab. Invest., **21,** 89-91 (1968).
9) ROSENBERG R.D. and DAMUS P.S., J. Biol. Chem., **248,** 6490-6505 (1973).
10) JORDAN R.E., OOSTA G.M., GARDNER W.T. and ROSENBERG R.D., J. Biol. Chem. **255,** 10081-10090 (1980).

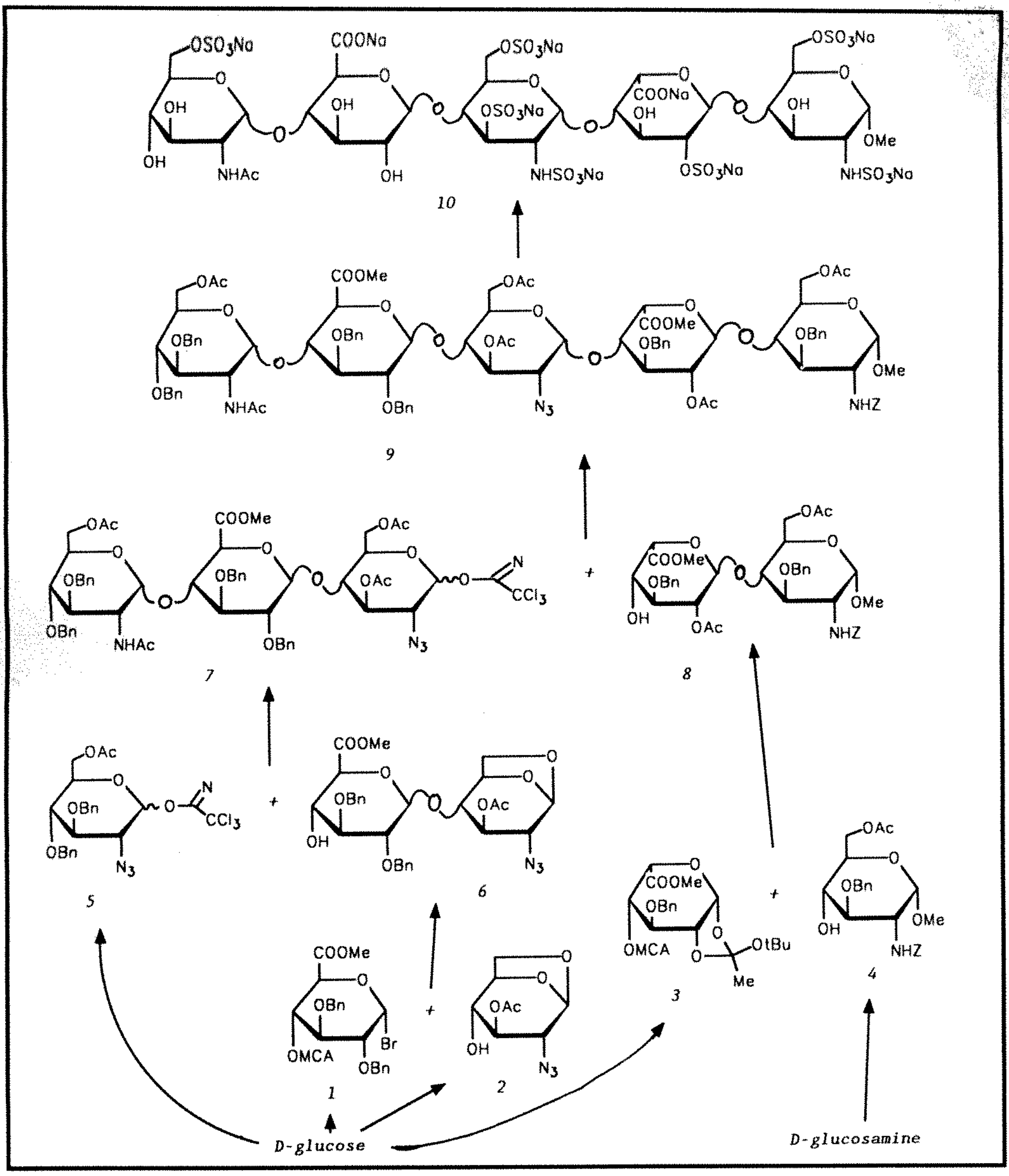


Figure 3: Chemical synthesis of a heparin-related pentasaccharide. The end product 10 corresponds to the sequence involved in the binding of porcine mucosa heparin to AT III (see figure 2). D-glucose and D-glucosamine are first converted into protected monosaccharides 1-5 bearing temporary (MCA:monochloroacetyl), semi-permanent (Ac:acetyl, Me:methyl) and permanent (Bn:benzyl) protective groups. Temporary protective groups are used for short term protection during chain elongation. Semi-permanent protective groups correspond to sulphate ester on the end product and are removed immediately before O-sulphation. Permanent protective groups are removed to give the hydroxyl functions of the end product. At the same time, azido and benzyloxycarboxylamino radicals yield amino functions of the glucosamines which are sulphated in the last step of the procedure. See reference 24.

3-O-sulphate in the central glucosamine unit is also remarkable: removal of this group completely abolishes AT III binding[30,31]. Other sulphate groups in the pentasaccharide play a similarly critical role while others contribute only to optimizing the interaction with the protein[32] (see table 1). This demonstrates how the chemical synthesis has made it possible to determine the role of different structural features in the binding of heparin to AT III.

Although animal studies show that synthetic pentasaccharides have suitable antithrombotic properties[33], there are several practical problems to be overcome in producing new antithrombotic drugs. It is not yet clear that they will work in humans and or that it will be possible to develop an industrial-scale production process.

The product of the current research will be improved antithrombotic drugs: in the meantime it has provided scientists with valuable tools for investigating the action of AT III and heparin, and the pharmacological properties of selective inhibitors of blood coagulation factor Xa.

11) ANDERSSON L.O., BARROWCLIFFE T.W., HOLMER E., JOHNSON E.A. and SIMMS G.E.C., Thromb. Res. 9, 575-583 (1976).
12) LAM L.H., SILBERT J.E. and ROSENBERG R.D., Biochem. Biophys. Res. Commun. 69, 570-577 (1976).
13) HÖÖK M., BJÖRK I., HOPWOOD J. and LINDAHL U., FEBS Lett. 66, 90-93 (1976).
14) LANE D.A., DENTON J., FLYNN A.M., THUNBERG L. and LINDAHL U., Biochem J. 218, 725-732 (1984).
15) WESSLER S. and YIN E.T., Thromb. Diathes. Haemorrh. **32,** 71-78 (1974).
16) KAKKAR V.V., DJAZAERI B., FOK J., FLETCHER M., SCULLY M.F. and WESTWICK J., Brit. Med. J. **284,** 375-379 (1982).
17) LORMEAU J.C., GOULAY J. and CHOAY J., French Patent Application 7831357 (06 Nov. 1978).
18) CHOAY J., LORMEAU J.C. PETITOU M., SINAŸ P. and FAREED J. Ann. NY Acad. Sci. **370,** 644-649 (1981).
19) CASU B., ORESTE P., TORRI G., ZOPPETTI G., CHOAY J., LORMEAU J.C. PETITOU M. and SINAŸ P., Biochem. J. **197,** 599-609 (1981).
20) THUNBERG L., BÄCKSTRÖM G. and LINDAHL U., Carbohydr. Res. **100,** 393-410 (1982).
21) For a review on this topic, see: BJÖRK I. and LINDAHL U., Mol. Cell. Biochem. **48,** 161-182 (1982).
22) PETITOU M., JACQUINET J.C., DUCHAUSSOY P., LEDERMAN I., CHOAY J. and SINAŸ P. in Glycoconjugates. Proceedings of the 7th International Symposium on Glycoconjugates, p.379 (1983).
23) CHOAY J., PETITOU M., LORMEAU J.C., SINAŸ P., CASU B. and GATTI G., Biochem. Biophys. Res. Commun **116,** 492-499 (1983).
24) For a review, see: PETITOU M. in Heparin, LANE D.A. and LINDAHL U. Eds., Edward Arnold, London, pp.65-79 (1989).
25) SINAŸ P., JACQUINET J.C., PETITOU M., DUCHAUSSOY P., LEDERMAN I., CHOAY J. and TORRI G., Carhohydr. Res. **132,** C5-C9 (1984).
26) PETITOU M., DUCHAUSSOY P., LEDERMAN I., CHOAY J. SINAŸ P., JACQUINET J.C. and TORRI G., Carbohydr. Res. **147,** 221-236 (1986).
27) VAN BOECKEL C.A.A., BEETZ T., VOS J.N., DE JONG A.J.M., VAN AELST S.F., VAN DEN BOSCH R.H., MERTENS J.M.R. and VAN DER VLUGT F.A., J. Carbohydr. Chem. **4,** 293-321 (1985).
28) ICHIKAWA Y., MONDEN R. and KUZUHARA H., Tetrahedron Lett. **27,** 611-614 (1986).
29) DUCHAUSSOY P., LEI P.S., PETITOU M. SINAŸ P., LORMEAU J.C. and CHOAY J., Bio. M. Chem. Lett. in press.
30) PETITOU M., DUCHAUSSOY P., LEDERMAN I. CHOAY J. and SINAŸ P., Carbohydr. Res. **179,** 163-172 (1988).
31) ATHA D.H., LORMEAU J.C., PETITOU M., ROSENBERG R.D. and CHOAY J., Biochemistry **24,** 6454-6461 (1987).
32) PETITOU M., LORMEAU J.C. and CHOAY J., Eur. J. Biochem. **176,** 637-640 (1988).
33) WALENGA J., PETITOU M., LORMEAU J.C., SAMAMA M., FAREED J. and CHOAY J., Thromb. Res. **46,** 187-198 (1987).
34) PETITOU M., DUCHAUSSOY P., LEDERMAN I., CHOAY J., JACQUINET J.C., SINAŸ P. and TORRI G., Carbohydr. Res. **167,** 67-75 (1987).
35) VAN BOECKEL C.A.A., BEETZ T. and VAN AELST S.F., Tetrahedron Lett. **29,** 803-806 (1988).
36) PETITOU M., JAURAND G., DERRIEN M., DUCHAUSSOY P. and CHOAY J., Bio. M. Chem. Lett., in press.
37) ATHA D.H., LORMEAU J.C., PETITOU M., ROSENBERG R.D. and CHOAY J., Biochemistry **24,** 6723-6729 (1985).
38) TEIEN A.N. and LIE M., Thromb. Res. **10,** 399-410 (1977).
39) KOMIYAMA Y., PEDERSEN A.H. and KISIEL W., Biochemistry **29,** 9418-9425 (1990).

- BETWEEN PASSION AND WISDOM, WHICH WOULD YOU CHOOSE ?

- PASSION.
BECAUSE PASSION IS OUR WISDOM.

The 90,000 men and women who work for ELF AQUITAINE have one point in common: the passion that drives them. They belong to a long line of discoverers. And it's thanks to this pioneering spirit that ELF AQUITAINE has become [illegible] competitive force in the international marketplace over the pa thirty years. A force in hydrocarbons. And force in chemicals and health sectors. Th present contract for oil exploration an production in the Soviet Union is indicative our dynamic spirit in action. ■

elf aquitaine

PASSION IS OUR WISDOM.